FACTORS LIMITING THE EXERCISE TOLERANCE OF PATIENTS WITH END-STAGE RENAL FAILURE UNDERGOING MAINTENANCE HAEMODIALYSIS

by

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BSc Physiotherapy, BSc (Med)(Hons) Sport Science

Thesis submitted for the degree of

Doctor of Philosophy (Medical Physiology)

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May 1994

DECLARATION

I, *WAYNE JONATHAN DIESEL*, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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DEDICATION

To my wife, Jean, thank you for your love and for blessing our home with two of the dearest children possible, Nicholas and Christine. I love you.

Cape Town

April, 1994

ACKNOWLEDGEMENTS

I wish to acknowledge and express my sincere appreciation to the following people

Professor Tim Noakes for all your attention to detail, teachings, countless corrections and tremendous insight. It has been the highlight of my academic career. A very special thank you for your endless support and friendship.

Professor Geoff Rogers, for the encouragement and guidance in the initial stages of this thesis. Thank you for the numerous hours you sacrificed assisting with Chapter 6. Also to your department, the Department of Physiology of the University of the Witwatersrand, for allowing me full use of their equipment and expertise.

Dr Charles Swanepoel, for your knowledge and friendship. Thank you for supporting me in my work as well as your continuous enthusiasm in helping me complete my research. A special thank you for performing all the muscle biopsies.

Dr Mike Lambert, for your patience and careful instruction of the testing procedures. Without your input this thesis would not have got off the ground.

Dr Larry Margolius, for your supervision whilst I was working with you in the Renal Unit of the Johannesburg Hospital.

The countless people who offered advice, support and direction when I needed it most.

Finally, the patients who willingly took part in this study, despite their tremendous illnesses. My thanks to you and your families. It is hoped that through this work, the total rehabilitation of this special group of patients can be improved.

ABSTRACT

Exercise tolerance, measured as peak oxygen consumption (VO_2 peak), is very low in patients with end-stage renal failure undergoing maintenance haemodialysis. Due to their associated anaemia and low peak heart rates during maximal exercise it has been argued that the reduced blood oxygen carrying capacity and central cardiovascular limitations are primarily responsible for the poor exercise tolerance of these patients. However, others suggest that peripheral (skeletal muscle) limitations including impaired substrate utilization, muscle weakness caused by peripheral neuropathy and myopathy, malnutrition and general physical deconditioning are responsible for the poor exercise tolerance. The present thesis was therefore designed to study whether central cardiovascular function or anaemia or muscle weakness causes patients with end-stage renal failure to terminate exercise at workrates well below those achieved by healthy controls.

The first study was designed to investigate the relationship between VO_2 peak and variables relating to either isokinetic skeletal muscle function or blood oxygen carrying capacity in patients with end-stage renal failure undergoing maintenance haemodialysis. VO_2 peak was measured during a maximum exercise test using a stationary cycle ergometer. Skeletal muscle function was measured using an isokinetic cycle ergometer and a Cybex II isokinetic dynamometer. There was a significant relationship between isokinetic

muscle strength and VO_2 peak but not between haemoglobin concentration and VO_2 peak. Therefore in renal haemodialysis patients, isokinetic muscle strength was a better predictor of exercise tolerance than variables determining blood oxygen carrying capacity.

Recent human studies in which partial correction of the anaemia associated with end-stage renal failure was achieved with human recombinant erythropoietin (EPO), have shown that this therapy causes a significant increase in the VO_2 peak of these patients. Several authors have interpreted these findings as further evidence for a central cardiovascular limitation to maximal exercise in these patients.

The second study was therefore designed to determine how EPO therapy alters physiological variables during submaximal and maximal exercise in patients with end-stage renal failure undergoing maintenance haemodialysis. Furthermore, the effect of EPO therapy on skeletal muscle function measured with an isokinetic dynamometer was studied. Haemoglobin concentrations and VO_2 peak rose significantly with treatment. However, neither submaximal oxygen consumption nor blood lactate concentrations at exhaustion were influenced by EPO treatment. These findings plus the absence of a 'plateau' in oxygen consumption during maximal exercise indicate that peripheral oxygen supply was probably not the most important factor limiting maximal exercise in these patients either before or after EPO treatment. But peak isokinetic muscle strength improved significantly with

EPO therapy. Thus the improved exercise tolerance of patients with end-stage renal failure who receive EPO therapy, does not appear to be solely due to reversal of a postulated muscle hypoxia which develops during maximal exercise but could result from an effect of EPO therapy on other peripheral factors, including some that might relate to skeletal muscle contractile function.

The objective of the third study was to determine whether patients with end-stage renal failure undergoing maintenance haemodialysis show morphological features suggestive of myopathic changes in the skeletal muscles of their lower limbs. Eight patients receiving chronic haemodialysis for end-stage renal failure consented to have open skeletal muscle biopsies performed. All of these patients had taken part in the first study and were therefore known to have poor exercise tolerance and skeletal muscle weakness. Tissues were examined by routine light and transmission microscopy. Fibre typing and sizing was quantitatively performed using computer-assisted morphometry. These values were within the normal population range and were not different from the controls. However, significant changes were found under light microscopy. These included fibre splitting, internalized nuclei, nuclear knots, moth eaten fibres, fibre degeneration and regeneration, increased content of lipid droplets and fibre-type grouping. Electron microscopy showed a large variety of non-specific abnormalities including mitochondrial changes, z-band degeneration, myofilament loss and accumulation of intra-

cellular glycogen. This study, although unable to offer an explanation for the pathogenesis of the myopathy, clearly demonstrated morphological changes in the muscle which may be sufficiently severe to account for the muscle weakness and severely impaired exercise tolerance found in all of these patients.

Hypertension and anaemia, both of which occur commonly in patients with end-stage renal failure undergoing maintenance haemodialysis, are often not controlled by adequate haemodialysis or blood transfusions, respectively. Atenolol is often the treatment of choice for the hypertension in patients receiving chronic haemodialysis. EPO partially corrects the anaemia associated with end-stage renal failure. Both forms of treatment are capable of influencing exercise tolerance during maximal exercise.

Therefore, the final study investigated the possible effects of non-selective grouping of patients, who are either on EPO therapy or ingesting beta-blockers on the interpretation of studies of exercise tolerance in patients with end-stage renal failure receiving maintenance haemodialysis. The study found that patients undergoing chronic haemodialysis and receiving Atenolol, 100mg daily, had $VO_{2\ peak}$ values and peak heart rates that were lower than values measured in control patients, without changes in peak isokinetic quadriceps muscle strength. Patients receiving EPO therapy and whose haemoglobin concentrations were similar to patients not requiring correction of their initially higher

haemoglobin concentrations, achieved comparable VO_2 peak values to those control patients.

This study therefore questions the conclusions drawn by other studies, which have included patients on beta-blockers in their total sample without appreciating that the low peak heart rates measured in those patients and which were assumed to have limited the exercise tolerance of these patients, may have resulted from their use of ß-blockers.

The results of these four studies lead us to conclude that impaired skeletal muscle contractile function or impaired skeletal muscle recruitment or both, may be important, but currently underappreciated, factors responsible, in part or whole, for the low exercise tolerance found in patients with end-stage renal failure undergoing maintenance haemodialysis.

A central cardiovascular limitation cannot fully explain the low exercise tolerance of these patients because the low peak lactate concentrations and absence of a 'plateau' in oxygen consumption during maximal exercise are not compatible with this interpretation. However, neither can skeletal muscle weakness fully explain the low peak heart rates and low peak rates of ventilation achieved by these patients as exercising heart rates and rates of ventilation are usually increased abnormally in persons with myopathy. It may, therefore, be possible that impaired recruitment of skeletal muscle fibres, resulting from either a central

motor dysfunction or peripheral neuropathy, may be another factor effecting the exercise tolerance of patients with end-stage renal failure undergoing maintenance haemodialysis during prolonged maximal exercise.

These findings indicate that in order to optimise the rehabilitation potential of patients with end-stage renal failure undergoing maintenance haemodialysis, therapeutic interventions that aim to correct potential peripheral contributors to exercise intolerance, such as skeletal muscle weakness and impaired recruitment of skeletal muscle fibres, must be included. Thus the low haemoglobin concentrations of these patients should not be considered the exclusive guideline for therapy nor should anaemia be considered the only factor requiring correction in these patients.

Further studies, possibly at the muscle cellular level, are necessary to understand the sequence of events responsible for the low exercise tolerance of patients with end-stage renal failure undergoing maintenance haemodialysis.

PUBLICATIONS AND ABSTRACTS

1. Diesel W, Noakes TD, Swanepoel C, Lambert M: Isokinetic muscle strength predicts maximum exercise tolerance in renal patients on chronic hemodialysis. Medicine and Science in Sports and Exercise 22(2):S30, April 1990.

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CHAPTER 1

INTRODUCTION

INTRODUCTION TO THE PROBLEM

Exercise tolerance, as measured by the peak oxygen consumption ($VO_{2\ peak}$) achieved during dynamic exercise using large muscle groups, is very low in patients with end-stage renal failure undergoing chronic haemodialysis [Barnea et al., 1980; Kettner et al.,1984; Painter et al., 1986(a); Zabetakis et al., 1982] and in those who have undergone successful renal transplantation [Painter et al., 1986(a); Kempeneers et al., 1990]. In one study only 60% of haemodialysis patients were capable of a physical working capacity beyond that required simply to care for themselves [Gutman et al., 1981].

The mechanisms responsible for the very low exercise tolerance of these patients has yet to be definitively identified. The extensive literature on this subject indicates that opinion is divided between those who believe that the main cause of the reduced exercise tolerance in these patients is the associated anaemia [Barnea et al., 1980; Kettner, 1982; Zabetakis et al., 1982; Zanconato et al., 1990; Lundin et al., 1991] causing impaired oxygen delivery to the active muscles [Moore et al., 1993]. In contrast, others believe that impaired muscle contractile function associated with the renal failure is the primary factor [Kettner-Melsheimer et al., 1987; Robertson et al., 1990].

Hence opinion is divided between those who believe (i) that either a central limitation of oxygen delivery to muscle resulting from the anaemia, or an associated impairment in oxygen uptake by the active muscles [Moore et al., 1993] explains the poor exercise tolerance of these patients, and those who favour (ii) impaired skeletal muscle contractile function, separate from both the anaemia and the postulated impairment in oxygen uptake by the active muscles.

STATEMENT OF THE PROBLEM

Measurement of peak oxygen consumption (VO_2 peak) has been used almost exclusively to assess exercise tolerance in patients with end-stage renal failure. Earlier studies concluded that because these patients were anaemic and their VO_2 peak were low, the reduced haemoglobin concentration must be the major factor limiting their exercise tolerance [Barnea et al., 1980; Kettner, 1982]. Accordingly, it was concluded that as the anaemia could not be improved in the long term without administration of either blood products or erythropoietin, nothing of significance could be done to improve the poor exercise tolerance of these patients. However, subsequent studies using recombinant human erythropoietin (EPO) or blood transfusions, both of which increased haemoglobin concentrations, showed that these interventions improved the exercise tolerance of these patients [Graf et al., 1987; Mayer et al., 1988; Clark et al., 1988; Canadian EPO Study Group, 1990]. These findings could support the interpretation that the anaemia was the

principle cause of the poor exercise tolerance of these patients.

However, closer inspection of these studies showed that the increase in haemoglobin concentration did not necessarily correlate with the improvements in VO_2 peak [Lundin et al., 1991; Metra et al., 1991].

Another finding which cast doubt on this theory of a central cardiovascular limitation for exercise tolerance in these patients was the low peak blood lactate concentrations achieved by these patients during maximal exercise [Sagiv et al., 1991]. In a recent review by Katz et al. (1990), it was concluded that exercise under conditions of impaired oxygen supply should result in increased blood lactate concentrations. Thus if their exercise tolerance is limited by impaired oxygen delivery to, or oxygen uptake by the active muscles, peak blood lactate concentrations in patients with end-stage renal failure undergoing chronic haemodialysis should be higher than values found in normal healthy subjects during maximal exercise.

In contrast, these patients achieve VO_2 peak values that are approximately 50% of age predicted values [Barnea et al., 1980] with peak blood lactate concentrations of only ± 5 $mmol.l^{-1}$ [Roseler et al., 1980; Sagiv et al., 1991]. If, as suggested, oxygen supply limits peak exercise in these patients then their peak blood lactate concentrations should be much higher. Hence, the peak blood lactate levels found

in these patients, although significantly above resting values, are too low to explain why they terminate exercise at such low workloads [Sagiv et al., 1991]. This suggests that aerobic energy production during maximal exercise in patients with end-stage renal failure undergoing chronic haemodialysis, may not be the most important factor limiting their exercise tolerance. Similar findings have also been found in patients after undergoing either renal or cardiac transplantation [Kempeneers et al., 1990; Kavanagh et al., 1979].

Robertson et al. (1990), quantified the improvement in exercise tolerance produced by EPO therapy in anaemic patients with end-stage renal failure, who were undergoing chronic haemodialysis. These authors suggested that since the patients' mean VO_2 peak improved by only 17%, despite a 64% improvement in haematocrit and arterial oxygen content, some as yet unidentified factor besides oxygen transport to tissues was the major factor limiting their peak exercise performance. Furthermore, Robertson et al. (1990) found quadriceps muscle strength, measured using a Cybex II Isokinetic Dynamometer (Lumex Corp, Ronkonkoma, NY), to be the single factor that best correlated with the VO_2 peak of these patients ($r = 0.65$). Thus they concluded that muscle weakness may be an important determinant of VO_2 peak in patients with end-stage renal failure undergoing chronic haemodialysis.

HYPOTHESIS

The hypothesis tested in this thesis is that skeletal muscle contractile factors are an important contributor to the poor exercise tolerance of patients with end-stage renal failure undergoing chronic haemodialysis.

SCOPE OF THIS STUDY

The purpose of this thesis was, therefore, to investigate the relative roles that both anaemia and muscle weakness played in the low exercise tolerance of patients with end-stage renal failure undergoing chronic haemodialysis.

The first study was designed to determine whether skeletal muscle function, measured as isokinetic muscle strength, or blood oxygen carrying capacity, measured as total blood haemoglobin content, better predicted peak oxygen consumption in patients with end-stage renal failure receiving chronic haemodialysis. It was concluded from this study that muscle strength was highly associated with the poor exercise tolerance in these patients.

In the second study, a group of anaemic patients with end-stage renal failure undergoing chronic haemodialysis were treated with EPO. This study showed that both VO_2 peak and isokinetic muscle strength improved with EPO therapy. It was concluded that EPO therapy enhanced the exercise tolerance of these patients by mechanisms not solely related

to an increased haemoglobin concentration and hence increased oxygen delivery to the active muscles. The finding that isokinetic muscle strength increased following EPO treatment is compatible with the interpretation that skeletal muscle function influences the exercise tolerance of these patients, regardless of their haemoglobin concentrations.

The above findings led to a study of the effects of chronic renal failure on skeletal muscle morphology in patients with end-stage renal failure undergoing chronic haemodialysis. Therefore, the third study described the morphological features of the myopathy associated with chronic renal failure. It was found that the muscles of patients undergoing chronic haemodialysis for chronic renal failure show significant morphological abnormalities. The severity of these changes suggest that they might explain the impaired skeletal muscle function of these patients.

The fourth study evaluated the effects of the use of medications commonly prescribed in patients with hypertension, including the beta-receptor antagonists, on the exercise tolerance of patients with end-stage renal failure. These agents are known to impair the exercise tolerance of normal, healthy subjects.

Thus the fourth study was designed to investigate the effects that various medications such as the beta-blocker receptor agonist, atenolol, and EPO have on the exercise

tolerance of patients with end-stage renal failure undergoing chronic haemodialysis.

It was found that exercise tolerance, as well as peak heart rates, of the patients receiving atenolol were significantly lower than values in patients not receiving this agent. Furthermore, no differences in exercise tolerance nor muscle strength were found between patients on maintenance EPO therapy and patients not on EPO but with similar haemoglobin levels.

Results from this study suggest that patients with end-stage renal failure undergoing chronic haemodialysis and who are receiving beta-blockers should not be included in studies investigating factors explaining the poor exercise tolerance of this group of patients. Inclusion of these patients in exercise-related studies may well reduce the mean peak heart rates and exercise tolerance of the total group by mechanisms unrelated to the primary disease. Since several studies cite low peak heart rates as evidence for a central (cardiovascular) limitation to peak exercise in these patients, the exclusion of patients on beta-blocker therapy from these exercise studies may be important and could alter the conclusions drawn in some of those studies.

In summary, since anaemia is characteristic of end-stage renal failure, it has always seemed reasonable to assume that reduced oxygen delivery to the active muscles during dynamic exercise must be the most important factor limiting

the exercise tolerance of these patients. Furthermore, the finding that EPO which partially corrects the anaemia associated with end-stage renal failure, also improves the exercise tolerance of these patients, appeared to support the oxygen-deficiency theory.

However, the studies undertaken in this thesis suggest that impaired skeletal muscle function may have an important role to play in contributing to the premature termination of exercise tolerance of patients with end-stage renal failure undergoing chronic haemodialysis. The important findings in these studies which support this conclusion include:
(i) the significant correlations between VO_2 peak and skeletal muscle strength measured isokinetically;
(ii) improvement of both skeletal muscle function and VO_2 peak after EPO therapy;
(iii) EPO therapy did not increase oxygen consumption during submaximal exercise even at the maximal work-load achieved before EPO therapy. Hence an increased oxygen supply to the active muscles after EPO therapy cannot explain why patients were able to continue to a higher maximal work-load;
(iv) severe morphological abnormalities are present in the skeletal muscle of these patients; and
(v) heart rates, rates of ventilation and most importantly, blood lactate concentrations are low at peak exercise in these patients.

It would be an extreme oversimplification to suggest that muscle weakness alone explained all of our findings. The

multisystemic pathologies, as outlined in Fig 2.1, must surely all contribute towards the low exercise tolerance of patients with end-stage renal failure undergoing maintenance haemodialysis. The autonomic dysfunction in haemodialysis patients is very likely to contribute to the low peak heart rates recorded in these patients. Whilst it is true that muscle weakness may prevent these patients from reaching higher peak blood lactate concentrations, it is equally possible that the reduced activities of key glycolytic enzymes and the metabolic acidosis restrict the lactate production in these patients. Furthermore, the skeletal muscle morphological abnormalities present in these patients may be the result of metabolic dysfunction. It is possible that EPO therapy partially corrects the metabolic dysfunction which may inturn improve skeletal muscle function.

Each patient, who voluntarily consented to participate in the various studies, underwent a full medical examination by a senior renal physician prior to testing. All were on stable medical regimens, diets, and haemodialysis programmes involving a 12 $hr.week^{-1}$ haemodialysis regimen. PCR (protein clearance ratio) and $KT.V^{-1}$ (K = constant; T = duration of haemodialysis; and V = blood volume of patient) were not yet being used to assess adequacy of dialysis. However, the clinical condition was regularly monitored to assess adequacy of dialysis.

LIMITATIONS OF THIS STUDY

The greatest limitation in this study was the availability of patients. Factors affecting the availability of suitable patients included "Third World" conditions such as lack of transport for the patients; poor communication caused by language differences; insufficient funds for treatment facilities or research; as well as unfamiliarity of exercise testing procedures. As EPO was only being introduced to South Africa at the time of this study, we were able to administer the drug to only 5 of the patients, one of whom did not wish to be part of the study. In order to get the sample size for these studies it was necessary to use patients from 4 major provincial hospitals. Since these hospitals are not in reasonable traveling distance from one another I had to relocate on two occasions. No objective scoring system was developed to test the relationship between skeletal muscle morphological abnormalities and skeletal muscle function. Such a scoring system would have improved the scientific merit of this thesis.

CHAPTER 2

REVIEW OF THE LITERATURE

Most patients with end-stage renal failure are entered into a renal dialysis programme before they undergo renal transplantation. Lack of donors or tissue incompatibility often means that patients must wait several years before undergoing successful renal transplantation. A number of studies have indicated that these patients should be encouraged to commence a regular programme of moderate physical activity as soon as possible in order to optimize their rehabilitation potential [Goldberg, 1984; Shalom et al., 1984; Zabetakis et al., 1982; Carney et al., 1987; Goldberg et al., 1983; Hagberg et al., 1983].

These studies have reported several important benefits of regular exercise including increased exercise tolerance sufficient to allow the patient to cope with a wider range of daily activities [Shalom et al., 1984], reductions in blood pressure so that patients require lower doses of anti-hypertensive medications [Hagberg et al., 1983], reduced feelings of depression leading to increased performance of pleasant activities [Carney et al., 1987] and a rise in haemoglobin concentrations in the absence of plasma volume changes [Goldberg et al., 1980].

The factors limiting the exercise tolerance of renal patients, who are either on dialysis or who have undergone successful kidney transplantation, need to be clarified before safe and effective exercise can be prescribed. Prescription of exercise, as in any clinical setting, should meet 3 fundamental criteria:-

(a) Assure regular participation in physical activity;

(b) Ensure that the chosen physical activity will not harm the patient (for example, contact sports are not acceptable since damage caused by an external force may easily damage a dialysis `access site' or transplanted kidney);

(c) Maximise the potential to increase functional capacity and health status.

Programme flexibility is needed so that the limitations imposed by the disease can be accommodated. Each category of renal patient, therefore, requires a programme specially tailored to their specific requirements and which encompasses realistic goals.

FACTORS AFFECTING THE EXERCISE TOLERANCE OF HAEMODIALYSIS PATIENTS

Patients with end-stage renal failure requiring chronic haemodialysis have poor exercise tolerance, usually measured as a reduced peak oxygen consumption ($VO_{2\ peak}$) [Barnea et al., 1980; Kettner et al.,1984; Painter et al., 1986(a); Zabetakis et al., 1982]. However, the predominant mechanisms causing this impaired exercise tolerance, which

may also contribute to a reduced level of habitual physical activity, are a matter of debate.

Determinants of physical performance include the capacity for skeletal muscle energy production (aerobic and anaerobic processes), neuromuscular function (muscle strength, co-ordination and technique), joint mobility and psychological factors (for example, motivation and self-discipline) [Astrand et al., 1977].

(i) Capacity for Aerobic and Anaerobic Energy Production

The conventional explanation for the low exercise tolerance of these patients has centered on the belief that oxygen supply to, or utilization by the active muscle mass, or both, is inadequate during exercise especially of near maximal intensity [Painter et al., 1987; Moore et al., 1993]. Accordingly, measurement of the $VO_{2\ peak}$ has been used frequently to estimate exercise tolerance in haemodialysis patients [Barnea et al., 1980; Painter et al., 1986(a); Zabetakis et al., 1982; Painter et al., 1987].

Aerobic Energy Production

Aerobic energy production is dependent upon the supply and utilization of oxygen. Thus it is important to understand the relationships which exist between the variables which control oxygen uptake.

The Fick principle [Little, 1985], which relates VO_2 (oxygen uptake ~ ml $O_2.min^{-1}$) at any workrate to the cardiac output

($CO \sim L.min^{-1}$) and the arteriovenous oxygen difference ($D(a-v)O_2 \sim ml\ O_2.ml^{-1}$ of blood), can be expressed as follows:

$$VO_2 = CO \times D(a - v)O_2 \quad \text{... eqn 1}$$

Equation 1 can be simplified by expressing cardiac output as a function of HR (heart rate $\sim$ $beats.min^{-1}$) and SV (stroke volume $\sim$ $ml.beat^{-1}$) and arteriovenous oxygen difference as a function of Hb (haemoglobin $\sim$ $g.L^{-1}$) and $SaO_2 - SvO_2$ (arterial oxygen saturation - mixed venous oxygen saturation). Thus

$$VO_2 = HR \times SV \times 1.34Hb[SaO_2 - SvO_2] \quad \text{... eqn 2}$$

Hence, according to the Fick principle, factors directly involved in, but not necessarily limiting,aerobic energy production include cardiac output (CO) expressed as the product of heart rate (HR) and stroke volume (SV), haemoglobin concentration (Hb), and the difference between the percentage saturation of oxygen in arterial and venous blood, multiplied by a conversion constant of 1.34 $ml O_2.gHb^{-1}$.

It should also be remembered that cardiac output (CO) may be influenced by peripheral factors such as change in total peripheral resistance (TPR) according to the following equation:-

$$CO = BP.TPR^{-1} \quad \text{... eqn 3}$$

(BP = Blood Pressure)

These peripheral influences on cardiac output may be relevant because of the role of the kidneys in regulating blood pressure through effects on blood volume and release of vasoactive hormones such as renin, and which may therefore be altered in patients with end-stage renal failure.

Pulmonary Ventilation

The lungs provide the initial interface for gaseous exchange. Furthermore, minute ventilation (V_E) influences VO_2 according to the following equation:-

$$VO_2 = V_E[F_IO_2 - F_EO_2] \quad \ldots \text{ eqn 4}$$

F_IO_2 and F_EO_2 are the fractional concentrations of oxygen in inspired and expired air, respectively. It should be noted that V_E only effects VO_2 in extreme conditions and V_E and F_EO_2 alter accordingly to keep VO_2 constant.

Their is no evidence to suggest that impaired pulmonary ventilation may contribute to the low exercise tolerance of renal haemodialysis patients. However, pulmonary congestion, which should not be present in patients receiving adequate haemodialysis, may affect exercise tolerance [Painter et al., 1987] by reducing gaseous exchange in the lungs.

Arterial Oxygen Content

In healthy subjects, the highest oxygen uptake that an individual can achieve during dynamic exercise using large muscle groups is influenced by the oxygen content of the arterial blood [Ekblom, 1986]. Patients with end-stage renal failure almost always develop severe anaemia [Guyton AC, 1981]. Decreased haemoglobin concentrations can therefore negatively influence the arterial oxygen content and can therefore influence the capacity for aerobic energy production [Moore et al., 1993].

Red cell 2,3-diphosphoglycerate concentration is increased in anaemia [Ganong, 1989]. At rest, the extremely low haematocrit (average 20% to 25%) and consequently low oxygen content is partially compensated for by a rightward shift of the oxygen-haemoglobin dissociation curve caused by metabolic acidosis and increased 2,3-diphosphoglycerate concentration [Ganong, 1989] characteristic of end-stage renal failure. This right shift causes more oxygen to be released to the tissues as a result of the decreased affinity of haemoglobin for oxygen at low oxygen tensions [Ganong, 1989]. Whether or not this mechanism is able to compensate fully for the anaemia and so to cope with the increased demand for oxygen use during exercise, remains to be investigated [Painter et al., 1987].

Significant correlations between haematocrit and exercise tolerance and haemoglobin concentrations and peak oxygen

consumption have been found in children [Ulmer et al., 1978] and adults [Zabetakis et al., 1982] with end-stage renal failure. However other workers did not find a correlation between exercise tolerance and haematocrit [Barnea et al., 1980], or between VO_2 peak and haematocrit following renal transplantation [Painter et al., 1984], or when the haematocrit was increased following blood transfusions [Sill et al., 1972].

Recombinant human erythropoietin (EPO) which partially corrects the anaemia associated with end-stage renal failure, causes significant increases in exercise tolerance of haemodialysis patients, even in the absence of an exercise training programme [Mayer et al., 1988; Canadian Erythropoietin Group., 1990; Robertson et al., 1990]. These findings have led researchers to believe that the increased haemoglobin concentration improves peripheral oxygen availability thereby resulting in a significantly higher exercise tolerance of patients with end-stage renal failure [Graf, Mayer & Thum, 1987(abs); Mayer et al., 1988; Lundin et al., 1991]. The conventional interpretation of this finding would be the oxygen-deficiency or "anaerobic" theory of fatigue, which holds that low haemoglobin concentrations and low arterial oxygen content are the main cause of impaired exercise tolerance in patients receiving chronic haemodialysis.

Cardiac Output

It has been suggested that impaired myocardial function can decrease exercise tolerance in haemodialysis patients [Bornstein et al., 1975; Scheer et al., 1975; Uroaka et al., 1975; Bullock et al., 1984]. Cardiac output, a function of heart rate and stroke volume (see eqns 1 and 2) or blood pressure and total peripheral resistance (see eqn 3), is linearly related to whole body oxygen consumption. Therefore, abnormalities in either heart rate, stroke volume, or the regulation of blood pressure or total peripheral resistance may adversely affect the capacity for aerobic energy production and hence the exercise tolerance.

In patients with end-stage renal failure, resting cardiac output is typically increased [Ikram et al., 1983] due to anaemia and fluid overload [Mayer, 1989]. Impaired myocardial contractility shown by declining stroke volume and ejection fraction with rising end-systolic and end-diastolic volumes, has been described in these patients even at rest [Hampl et al., 1985]. Low left ventricular ejection fractions have also been correlated with low exercise tolerance in these patients [Bullock et al., 1984]. All these findings suggest a central, cardiac limitation to exercise tolerance in these patients.

Dysfunction of the autonomic nervous system resulting from the underlying uraemia [Mallamaci et al., 1986], may be responsible for the low peak heart rates measured during maximum exercise in patients with end-stage renal failure

undergoing haemodialysis [Painter et al., 1984]. Studies have demonstrated that despite comparable resting heart rates, haemodialysis patients have lower heart rates at similar work intensities, as well as lower peak heart rates, than do healthy sedentary controls [Kettner et al., 1984; Zabetakis et al., 1982; Painter et al., 1986(a)]. However, a lower peak heart rate is to be expected since these patients also terminate exercise at significantly lower peak workloads.

Impaired left ventricular function, possibly due to compromised contractile function or increased after-load due to hypertension, or both, will adversely affect stroke volume [Painter et al., 1987]. Negative inotropic states may result from hyperkalaemia, hypocalcaemia, hypermagnesaemia and acidosis [Ayus et al., 1981]; all of which are features of the associated uraemia. Increased left ventricular afterload may be caused by hypertension, anaemia, and shunting through the arteriovenous fistula [Capelli et al., 1977]. However, the arteriovenous fistula created for renal haemodialysis appears not to produce significant effects on heart rate, stroke volume, arterial pressure, and right atrial pressure at peak levels of exercise [Payne et al., 1972].

Thus physical activity in haemodialysis patients can be further compromised as a result of a reduced cardiac output, resulting both from a diminished heart rate response to exercise and a reduced stroke volume.

In addition, hypertension, associated with renal failure, has often been treated with beta-receptor antagonists (beta-blockers) in patients undergoing chronic haemodialysis [Friedman E, 1979]. The detrimental effects of beta-blockers on exercise performance in healthy subjects are well known; in particular there is a reduction of maximal heart rate and reduced time to fatigue during maximal exercise [Van Baak, 1988]. However, despite these well known ergolytic effects of beta-blockers, many authors studying the exercise tolerance of renal patients persist in grouping patients, irrespective of whether or not they are receiving beta-blockers, and drawing conclusions about the factors which might limit these patients' exercise capacities including their low peak heart rates [Painter et al., 1986(a); Kettner-Melsheimer et al., 1987; Moore et al., 1993].

Clearly these conclusions will be influenced by the nature of the medications used by the patients. Hence there is a need to exclude those patients, who are on medications that might either enhance or impair $VO_{2\ peak}$, from clinical trials designed to investigate factors limiting the exercise tolerance of these patients.

Muscle Blood Flow

Painter et al. (1987(a)) have proposed a hypothetical model which suggests that the metabolic acidosis and autonomic dysfunction associated with chronic renal failure may lead

to generalised arteriolar vasodilation which would result in a failure to redistribute blood flow away from non-working tissues during exercise. Thus they concluded that a reduced blood supply to the active muscle groups during exhaustive exercise may limit the maximal exercise capacity of patients with end-stage renal failure undergoing chronic haemodialysis.

However, since muscle blood flow in these patients, has yet to be measured during maximal exercise, no definite conclusions regarding reduced muscle blood flow during dynamic exercise can be drawn at present.

Substrate Utilization

During exercise both carbohydrate and fat are essential substrates for the production of energy [McArdle et al., 1981]. Several metabolic abnormalities affecting the utilization of carbohydrate and fat have been identified in haemodialysis patients [DeFronzo et al., 1981; Chan et al., 1981].

Blood glucose concentrations during exercise are normal in haemodialysis patients despite significantly higher concentrations of insulin and glucagon throughout exercise [Kettner et al., 1984]. Increased insulin concentrations inhibit lipolysis and stimulate muscle glucose uptake [Martin et al., 1981]. However, this action of insulin may be impaired because of higher plasma concentrations of glucagon and parathyroid hormone [Eigler et al., 1976], both

of which are elevated in patients receiving chronic haemodialysis [Bilbrey et al., 1974; Slatopolsky et al., 1973] and which both impede glucose uptake by muscle. Furthermore, inhibition of the rate-limiting glycolytic enzyme, phosphofructokinase [Renner et al., 1972], can further impair carbohydrate metabolism [Frolich et al., 1978], which may then ultimately contribute to the associated weakness and fatigue [Nakoa et al., 1982] by reducing the maximum capacity for glycolytic energy production in the active muscles.

Artificially induced acidosis in healthy sedentary subjects during exercise causes early fatigue by decreasing the rate of rephosphorylation of ADP from glycolysis [Sahlin et al., 1986]. Thus the metabolic acidosis present in end-stage renal failure [MacCleod J, 1977]and the reduced activity of the rate-limiting enzyme in the glycolytic pathway, phosphofructokinase [Renner et al., 1972; Metcoff et al., 1978], may contribute to the early fatigue experienced by these patients during exercise.

This argument is supported by the finding that the energy charge ratios in skeletal muscle at rest, as reflected by ($ATP.ADP^{-1}.AMP^{-1}$) ratios, were found to be significantly lower in haemodialysis patients compared to healthy sedentary controls [Cleminson et al., unpublished data].

This is possibly due to lower resting muscle phosphagen (ATP and PCr) concentrations in haemodialysis patients [DelCanale

et al., 1986] or reduced skeletal muscle mitochondrial oxidative capacity. There is also a delayed recovery of muscle phosphocreatine concentrations during maximal exercise in haemodialysis patients [Nishida et al., 1991].

Two important steps in the metabolic pathway for the oxidative metabolism of long-chain fatty acids may also be impaired in the skeletal muscles of patients receiving chronic haemodialysis [Siami et al., 1991; Smogorzewski et al., 1988].

Firstly the concentrations of L-carnitine, which plays an important role in mitochondrial beta-oxidation of long-chain fatty acids [Fritz, 1959] and is therefore important in energy production in skeletal muscle [Engel et al., 1973], are significantly lower in the skeletal muscles of patients with end-stage renal failure than in healthy controls [Bohmer et al., 1978; Leschke et al., 1983]. L-carnitine supplementation improved muscle endurance in a group of haemodialysis patients [Siami et al., 1991]. The mechanism explaining this effect is unclear. It is of interest that an abnormal distribution of lipid droplets in type I skeletal muscle fibres is considered to be the hallmark of the myopathy caused by carnitine deficiency [DiMauro et al., 1980]; yet no such abnormality has been observed in skeletal muscle biopsies from patients with chronic renal failure [Ahonen, 1981]. One possible explanation for the improved muscle endurance is that carnitine supplementation, in patients with end-stage renal failure undergoing maintenance

haemodialysis, reduces muscle catabolism [Ahmad et al., 1990].

Secondly, fatty acid oxidation by the skeletal muscles of renal patients is further impaired as a result of elevated parathyroid hormone concentrations with reduction in the activity of carnitine palmitoyl transferase [Smogorzewski et al., 1988], a key enzyme essential for the transport of long-chain fatty acids into the mitochondrial matrix for beta-oxidation in muscle [Banks et al., 1979].

Lactate Production

There is considerable disagreement in the literature regarding blood lactate concentrations during exercise in haemodialysis patients.

Thus it has been suggested that blood lactate concentrations are either relatively high in haemodialysis patients when compared to healthy sedentary controls [Parrish et al., 1981], or they are reduced during exercise [Nakoa et al., 1982] or they are no different to concentrations measured in normal subjects, at similar work intensities [Kettner et al., 1984].

Parrish et al. (1981) reported elevated blood lactate concentrations in patients receiving chronic haemodialysis compared to normal healthy subjects at the same absolute exercise intensity. This should not be surprising since the renal patients were exercising at higher relative exercise

intensities as indicated by their significantly higher heart rates at those workloads (101 ± 13 vs 81 ± 12 beats). The intensity of work relative to the individual's maximal exercise capacity determines the blood lactate concentrations [Wasserman et al., 1973]. Thus studies which suggest that haemodialysis patients have elevated blood lactate concentrations during exercise may have reached incorrect conclusions if the relative exercise intensities for the renal patients and for the controls were not standardized.

In contrast, other studies have suggested that the blood lactate response to exercise is diminished in patients with end-stage renal failure [Nakoa et al., 1982] and shows a similar response to that present in patients with McArdle's syndrome [Floyd et al., 1974]. Patients with McArdle's syndrome have a greatly reduced exercise tolerance; in addition these patients are unable to mobilize muscle glycogen to provide the energy for muscle contraction [Ganong, 1989, pg 243]; hence blood lactate concentrations of patients with McArdle's syndrome do not rise significantly above resting concentrations. Rather the oxidation of blood-borne glucose and free fatty acids provide the major sources of energy [Braakhekke et al., 1986]. This inability to utilize muscle glycogen results in its accumulation in the skeletal muscles of these patients [Ganong, 1989 pg 243]. These twin characteristics of a reduced exercise tolerance and the accumulation of muscle glycogen have also been reported in haemodialysis patients

[Floyd et al., 1974; Nakoa et al., 1982]. However, at peak exercise, patients with end-stage renal failure have blood lactate concentrations which are significantly higher than their resting concentrations [Röseler et al., 1980; Parrish et al., 1981; Kettner et al., 1984; Sagiv et al., 1991; Kempeneers et al., 1990].
Thus it is incorrect to equate the blood lactate response to exercise in patients with end-stage renal failure with that of patients with McArdle's syndrome.

The blood lactate concentrations at rest and at similar relative intensities of submaximal exercise have been reported to be similar in control subjects and in those with end-stage renal failure receiving chronic haemodialysis [Kettner et al., 1984]. Peak blood lactate concentrations measured in healthy sedentary subjects are reported to be in the region of 10 - 15 $mmol.L^{-1}$ [Harmansen et al., 1971], whereas peak blood lactate concentrations of haemodialysis patients are in the region of only 5.25 ± 1.67 $mmol.L^{-1}$ [Röseler et al., 1980; Sagiv et al., 1991]. These findings indicate that while blood lactate concentrations at rest and during submaximal exercise are similar in healthy controls and in patients with end-stage renal failure, peak concentrations are much lower in renal patients at the end of maximal exercise. A probable explanation for the lower peak blood lactate concentrations in these patients would be the much lower workloads that they achieve during maximal exercise.

Therefore elevated blood lactate concentrations and the associated rise in hydrogen ions, which influence many of the processes involved in the transformation of chemical energy into mechanical work in healthy subjects during maximal exercise [Sahlin, 1986], do not appear to be the factor responsible for the low exercise tolerance found in haemodialysis patients [Sagiv et al., 1991].

(ii) Neuromuscular Function

Uraemic Neuropathy

Nervous system dysfunction in the form of a peripheral neuropathy, remains a major cause of disability in patients with end-stage renal failure [Fraser et al., 1988] and is present in about 65% of patients who are being treated with haemodialysis [Lindblom et al., 1985]. The peripheral neuropathy is characterized by a mixed motor and sensory polyneuropathy, with a distribution that often results in weakness and wasting in the arms and legs [Nielsen, 1974].

Uraemic Myopathy

There is a progressive loss of muscle strength, most pronounced in the legs, with advancing renal failure. This does not appear to improve with haemodialysis [Kettner, 1982]. It has been suggested that the underlying uraemia and acidaemia associated with end-stage renal failure or the treatment of end-stage renal failure is responsible for the muscle weakness [Floyd et al., 1974]. Others have suggested

that the myopathy is a result of the secondary hyperparathyroidism associated with end-stage renal failure [Frans et al., 1989].

Wasting and Malnutrition

Undernutrition depresses skeletal muscle function in healthy subjects [Chan et al., 1986]. Patients with renal failure, even when on haemodialysis, are poorly nourished as a result of anorexia from uraemic toxicity, medications, superimposed illness, psychological depression and relatively unpalatable meals as well as oral and gastric abnormalities such as mucosal ulceritis, oesophagitis, ascites and colonic obstructions [Massry et al, 1989; Harvey et al., 1980]. It is therefore not surprising that wasting and malnutrition are common in renal patients and most probably contribute to the muscle weakness in these patients [Blumenkrantz et al., 1980; Berkelhammer et al., 1985; Kopple, 1983; Alvestrand et al., 1983].

The histopathology of this muscle weakness seen in renal failure is characterized by type II muscle fibre atrophy, identical to that observed in cachexia [Jennekens, 1981].

General Physical Deconditioning

General physical deconditioning, not necessarily due to strict bed rest, is known to reduce exercise tolerance. After three weeks of strict bed rest, $VO_{2\ max}$ decreased by 26% [Saltin et al., 1968]. Patients with end-stage renal failure generally lead extremely sedentary lifestyles. It

would not, therefore, be surprising if their inactivity also contributes to their poor exercise tolerance. Evidence for this suggestion is the increase in exercise tolerance associated with the introduction of exercise programmes of modest intensities [Shalom et al., 1984; Harter et al., 1985; Carney et al., 1987].

(iii) Psychological factors

Physical performance may be influenced by various psychological factors [Astrand et al., 1977]. Depression appears to be the most prevalent psychological reaction of patients undergoing chronic haemodialysis [Maher et al., 1983; Levy, 1984]. Other problems include dependency, denial, stress, low quality of life, sexual dysfunction and fear of dying [Brown et al., 1984]. These psychosocial factors may adversely affect compliance to exercise programmes [Painter et al., 1987]. The possibility that these factors might also limit physical performance and habitual activity has not yet been investigated but needs to be considered.

(iv) Conclusion

In conclusion, patients with chronic renal failure undergoing chronic haemodialysis are severely limited in both their habitual physical activity and in their exercise performance.

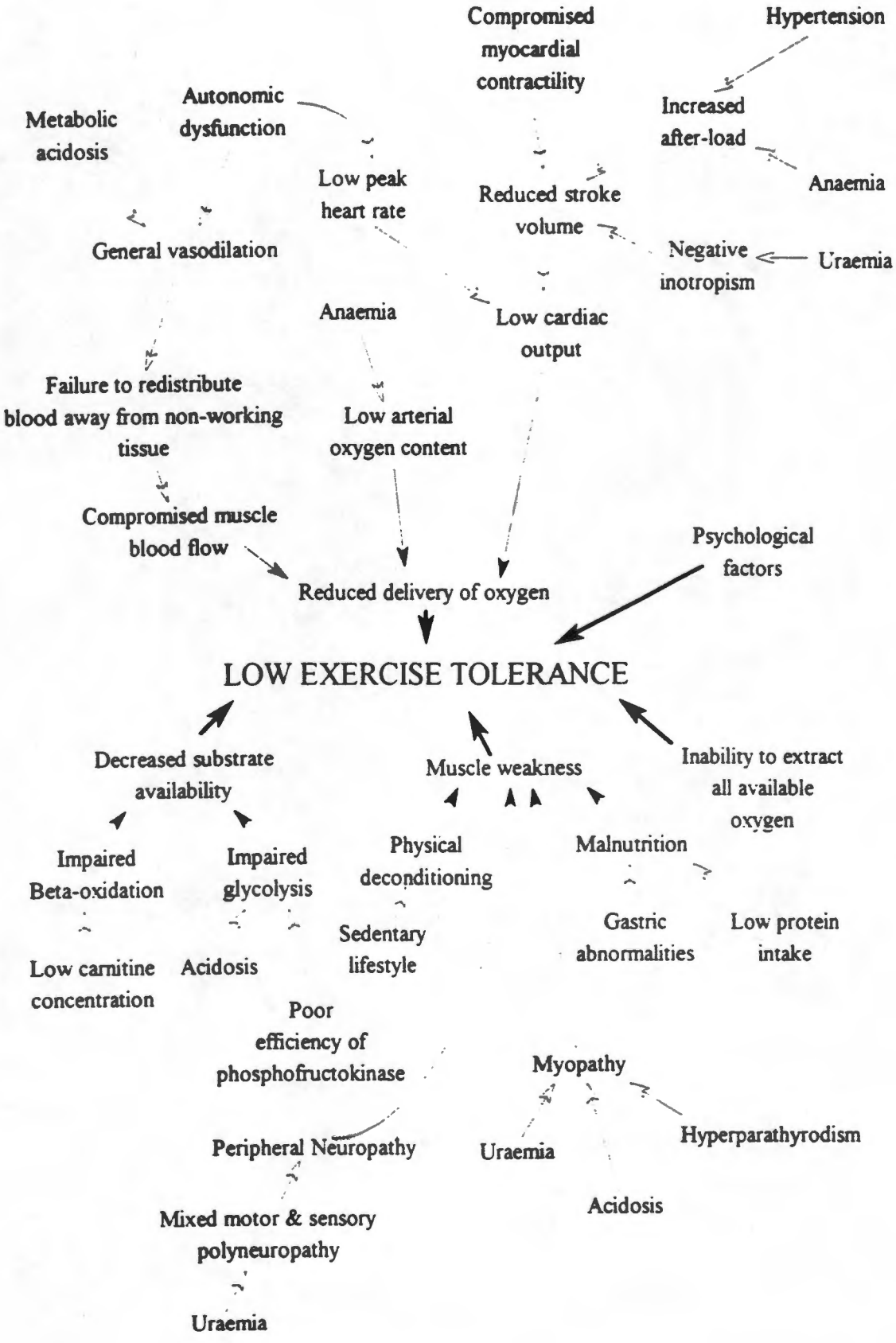

Figure 2.1 Possible factors limiting the exercise capacity of patients with end-stage renal failure receiving maintenance haemodialysis.

From the literature it appears as if opinion is divided into those who believe that the poor exercise tolerance of these patients results from central (cardiovascular) abnormalities including reduced haemoglobin concentrations, arterial oxygen content, cardiac output, muscle oxygen extraction capabilities, and a compromised blood flow to the working muscles (see Fig 2.1); and those who argue for peripheral (skeletal muscle) limitations including impaired substrate utilization, muscle weakness caused by peripheral neuropathy, myopathy, malnutrition and general physical deconditioning.

Recently Moore et al. (1993) have argued that impaired cardiovascular function together with anaemia and a reduced capacity for skeletal muscle oxygen extraction, limit the exercise tolerance of patients with end-stage renal failure receiving chronic haemodialysis. First they find that peak heart rates during maximum exercise are low in these patients, suggesting impaired central cardiovascular function; second even though arterial oxygen content was low because of the anaemia, the mixed venous oxygen content was the same, and the arterial-venous oxygen ($A\text{-}VO_2$) difference was less than values measured in other trained and sedentary subjects. When considered with the finding that EPO therapy does not always increase $VO_{2\ peak}$ and $A - VO_2$ difference during exercise, they argue that their second findings suggests that the muscles of patients with end-stage renal failure do not have a normal capacity for oxygen extraction. However Moore et al. (1993) have not shown that the reduced

heart rate response of these patients to exercise is due to a primary myocardial disease and secondly that the skeletal muscle contractile function of these patients is normal. On the contrary, skeletal muscle contractile function in patients with end-stage renal failure undergoing maintenance haemodialysis may be effected by conditions associated with the disease such as neuropathy [Kettner, 1982]; malnutrition [Berkelhammer et al., 1985]; secondary hyperparathyroidism [Smogorzewski et al., 1988]; and low carnitine muscle concentrations [Siami et al., 1991].

The emphasis of this thesis will be, therefore, to decide whether central cardiovascular function, or anaemia or muscle weakness causes patients with end-stage renal failure to terminate exercise at workloads well below those achieved by healthy controls. We planned to investigate the relationships between exercise tolerance, expressed as peak oxygen consumption, and variables relating to either skeletal muscle function or blood oxygen carrying capacity. Since the effects of EPO therapy on exercise tolerance appeared to support the anaemia argument, it was decided to study the effects and relationships that EPO had on exercise tolerance and skeletal muscle function. Furthermore, it was necessary to analyse the morphological features of their skeletal muscle to assess whether or not changes within the muscle structure may account for the muscle weakness and hence the severely impaired exercise tolerance found in these patients. Our final study was designed to determine the effects of beta-blocker therapy on peak heart rate values during maximal exercise in this group of patients.

CHAPTER 3

ISOKINETIC MUSCLE STRENGTH PREDICTS MAXIMUM EXERCISE TOLERANCE IN RENAL PATIENTS RECEIVING CHRONIC HAEMODIALYSIS

INTRODUCTION

The poor exercise tolerance of patients with chronic renal failure receiving haemodialysis is believed to be due, at least in part, to the associated anaemia which limits oxygen delivery to skeletal muscles especially during maximal exercise [Painter et al., 1987; Zabetakis et al., 1982; Barnea et al., 1980]. Thus Zabetakis et al. (1982) found a significant correlation between blood haemoglobin concentration and peak oxygen consumption during maximal exercise in renal patients receiving chronic haemodialysis. However others have either failed to show a relationship between variables of blood oxygen carrying capacity and exercise tolerance [Barnea et al., 1980; Lundin et al., 1981] or any increase in physical performance subsequent to correction of haematocrit by blood transfusion [Sill et al., 1972] or renal transplantation [Painter et al., 1986(a)]. Thus other factors such as altered peripheral metabolism, general physical deconditioning and ventricular dysfunction might better explain the impaired exercise tolerance of these patients [Painter et al., 1986(b); Lundin et al., 1987].

A characteristic feature of chronic renal failure is the associated myopathy [Floyd et al., 1974]. It would be surprising if this myopathy was without effect on the exercise tolerance of patients receiving chronic haemodialysis.

To determine whether the maximal exercise tolerance of patients receiving chronic haemodialysis is limited by skeletal muscle factors, we performed conventional maximum exercise tests for measurement of peak oxygen consumption (VO_2 peak) in ten adult renal haemodialysis patients. In addition, isokinetic muscle strength was measured with an isokinetic cycle ergometer and a Cybex Isokinetic Dynamometer designed to maintain pedal-crank and angular velocity, respectively, constant at a pre-set level regardless of the torque generated by the subject [McCartney et al., 1983; Hislop et al., 1967]. Peak power produced during maximal cycling exercise of short (10 seconds) duration is considered to be independent of oxygen delivery and is therefore a measure of oxygen-independent skeletal muscle power (muscle contractility) [Oldridge et al., 1989; McCartney at al., 1989]. Further advantages in the use of isokinetic muscle testing is that this method allows the measurement of maximal torque over a wide range of contraction velocities and joint angles. In addition, the measurements are reproducible and the technique has been found to be safe in other groups of chronically ill patients [Oldridge et al., 1989; McCartney at al., 1989].

The purpose of this study was therefore to determine whether skeletal muscle function measured as isokinetic muscle strength or blood oxygen carrying capacity measured as total blood haemoglobin content, better predicted peak oxygen

consumption and exercise tolerance in renal patients receiving chronic haemodialysis.

MATERIALS AND METHODS

Ten adult (7 female and 3 male) patients with chronic end-stage renal failure receiving chronic haemodialysis volunteered for the study which had been approved by the Ethics and Research Committee of the Faculty of Medicine of the University of Cape Town. Each patient underwent a full medical examination by the senior renal consultant of the haemodialysis unit at Groote Schuur Hospital, before being accepted on to the study. The medical status of all patients accepted onto the programme was considered to be stable and all were considered capable of completing the study. Patients were randomly assigned to either of two groups. Group A first completed a maximum exercise test to exhaustion for measurement of $VO_{2\ peak}$ and exercise tolerance; in the following week they performed the isokinetic cycle test for measurement of peak isokinetic power at different cycling cadences. This sequence was reversed for Group B.

After they had completed these tests, patients also performed maximal isokinetic exercise at two different contraction velocities on a Cybex II Isokinetic Dynamometer (Cybex, Ronkonkoma, NY) with a Cybex Data Reduction computer. Only maximum isokinetic torque of the quadriceps muscle group from the dominant limb was recorded at both

speeds. All testing was performed on a non-dialysis day, usually after the last haemodialysis treatment for that week.

Blood was drawn the day before the maximum exercise test for measurement of haemoglobin concentration and haematocrit using a model S-Plus Coulter counter (Coulter Electronics Inc., Miami, FL) in combination with an automatic dilution and mixing device for sample processing and a single-beam photometer for haemoglobin measurements [Brittin et al., 1971].

The following day, patients reported to the laboratory for measurement of peak oxygen consumption using an electronically-calibrated stationary cycle ergometer. Patients cycled with a model No. 2766 counterbalanced head support holding a model No. 2700 Rudolph valve (both by Hans Rudolph Inc., Kansas City, KA). A nasal clip prevented nasal breathing. Expired air was sampled and analyzed as previously described [Noakes et al., 1990].

In brief, expired air was continuously sampled from a 15 l perspex mixing chamber to the pick-up heads of an Ametek oxygen analyzer model S-3A1 (Applied Electro-Chemistry, Ametek Inc., Pittsburgh, Pennsylvania, USA), and an Ametek carbon dioxide analyzer model CD-3A (Applied Electro-Chemistry, Ametek Inc., Pittsburgh, Pennsylvania, USA). Both analyzers were calibrated before and after each test using gases of known composition. Inspiratory volume was

recorded with a Mijnhardt dry gas meter that had been calibrated against a Collins chain-compressed gasometer (Collins Inc., Braintree, Massachusetts, USA). Rates of oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratios (RER) were calculated each minute by an on-line computer (Apple) using locally developed software (A.R.T.) [Noakes et al., 1990] based on conventional equations [Jones Nl, 1988]. Exercise heart rates were measured with a Lohmeier M607 ECG monitor (Munich, West Germany), using self-adhering electrodes placed in the CM5 position. Blood pressure was measured with a portable mercury sphygmomanometer. The cuff was placed on the arm that did not have the arterio-venous fistula. Blood pressure was measured every two minutes during exercise.

Prior to commencement of the test, an intravenous catheter placement unit (Criticon; Tampa, FL, USA), was inserted into a subcutaneous forearm vein distal to the arterio-venous fistula and connected via pre-heparinized tubing to an Eyela Microtube pump (Rikakikai Co Ltd, Tokyo, Japan), which drew blood continuously at a rate of 2 $ml.min^{-1}$. Blood samples were collected in test tubes containing 2 ml of 0.6N perchloric acid (PCA) every minute for 30 seconds during the test and at three minutes after stopping. Blood samples were centrifuged and the supernatant stored at -4^{o}C pending analysis for lactate concentration by conventional techniques [Gutman et al., 1974].

The exercise test protocol involved progressive increments in workrate until the patients voluntarily terminated the test. Patients were allowed a four minute warm-up period during which they cycled against a workrate of 0 Watts. During this time they were familiarized with the apparatus used for collection of expired air samples. No measurements were recorded during this initial warm-up period. Thereafter they exercised for a further two minutes at a workrate of 0 Watts after which the workrate was increased by 15 Watts every 2 minutes until exhaustion. Patients were asked to identify the nature of the symptoms they experienced at exhaustion.

The isokinetic cycle ergometer is a modified ergometer with a strengthened frame designed according to that of McCartney et al., 1983. Special components include a 2.2kW DC electric motor (Femco 1605037), which serves as a dynamic load, a personal computer (Bondwell International, Hong Kong), and a locally designed Data Acquisition Unit (DAU) based on the Hitachi 6303 microprocessor and containing 8K ROM and 48K RAM.

Forces applied to the cranks are detected by four strain gauges. These strain gauges are calibrated by static loading with known weights on the left and right pedals which are locked in a forward facing horizontal position. Signals from the strain gauges are amplified by amplifiers located on the chain wheels. Peak signal values are recorded as peak torque during every cycle. The average

power per cycle is obtained by the trapezoidal rule. The work done per cycle is obtained directly from the result of the integral, that is the area under the torque.angle^{-1} curve, whereas average power is obtained by dividing the work done in a particular cycle by the duration of the cycle.

Prior to testing the height of the saddle was adjusted to the preferred height for the cyclist. A position which allowed slight knee flexion when the pedal crank was angled vertically downward was used as the optimum saddle height [McCartney et al., 1983].

Each patient completed three 10 second maximal bouts of cycling at 70, 90 and 110 revolutions per minute. Before beginning each test, patients performed a brief warm-up at the required crank velocity to familiarize themselves with the ergometer. Patients rested for 1 minute after the warm-up and for 2 minutes between the different tests.

Patients also performed isokinetic exercise on the Cybex Isokinetic Dynamometer at limb contraction velocities of 60 and 90 degrees.sec^{-1} [Hislop et al., 1967]. After they had performed 5 warm-up repetitions at each of the test speeds, patients completed six maximal repetitions at each speed. The warm-up was followed by a rest period of 1 minute. Patients rested for 2 minutes before beginning the warm-up at the next speed.

Statistical methods

Data are expressed as mean ±SD. Data for ventilation and blood lactate concentrations during exercise were fitted with the non-linear regression programme of the Institute for Scientific Information Graphpad (Philadelphia, Pennsylvania, USA). For all the other relevant data, straight lines were fitted by conventional least squares analyses. Statistical significance was assessed from the correlation coefficients ($p < 0.05$).

RESULTS

The anthropomorphic and haematological measurements in the 10 patients are listed in Table 3.1. The mean duration of haemodialysis was 4.4 ± 3.8 (Mean ±SD) years.

TABLE 3.1 ANTHROPOMORPHIC AND HAEMATOLOGICAL MEASUREMENTS AND CAUSE OF RENAL FAILURE IN 10 PATIENTS WITH END-STAGE RENAL FAILURE

Sex	Age (Yrs)	Height (m)	Weight (kg)	Cause of renal failure	Duration of dialysis (Yrs)	Hb ($g.dl^{-1}$)	Hct (%)
F	43	1.55	58.2	GN	3	8.3	26
F	55	1.59	57.0	HT	4	8.2	24
F	47	1.64	56.8	PK	2	7.5	23
M	26	1.64	62.2	GN	5	7.5	21
M	52	1.60	61.3	AN	8	7.8	23
F	51	1.52	51.8	HT	1	8.6	26
F	26	1.64	62.1	GN	13	9.1	26
F	37	1.55	45.1	HT	7	4.6	12
M	17	1.68	59.4	SLE	0.8	8.6	25
F	23	1.51	44.8	GN	0.4	11.0	30
Mean	38	1.60	56.5		4.4	8.1	24
±SD	±13	±0.06	±6.4		±3.8	±1.6	±4.7

Key: GN = glomerulonephritis; HT = hypertensive renal disease; PK = polycystic kidney; AN = analgesic nephropathy;
SLE = systemic lupus erythematosis.

Glomerulonephritis was the cause of the chronic renal failure in 4 (40%) patients. Other causes of renal failure included hypertensive renal disease (30%) and systemic lupus erythematosis, polycystic kidney and analgesic nephropathy (10% each). The mean haemoglobin concentration and haematocrit were 8.1 ± 1.6 $g.dl^{-1}$ and 24 ± 4.7,respectively.

One female patient, aged 37 years, had a haemoglobin concentration of 4.6 $g.dl^{-1}$ and a haematocrit of 12%. The patient had concentrations of cytotoxic antibodies in excess of 50%. In view of this and because her condition was stable at the time of this study, it was considered advisable that she not receive unnecessary blood transfusions at that time. Despite this, she had received 7 units of blood in the 12 months prior to the study. Despite having such a low haematocrit she completed all the testing procedures without any complications even though her VO_2 peak was only 13.7 $ml.kg^{-1}.min^{-1}$, equal to that of another patient with a haematocrit of 24% and a haemoglobin content of 8.2 $g.dl^{-1}$. The patient was included in the study because of her keen interest in participating and her initial medical examination which found her to be in a stable condition.

Table 3.2 lists the cardiorespiratory and metabolic variables measured at rest and during peak exercise. Resting heart rate was elevated (98 ± 21 $beats.min^{-1}$) and rose to 168 (± 12) $beats.min^{-1}$ during maximal exercise. Mean resting blood pressure was normal but 5 patients had

moderate hypertension (systolic blood pressure >145 mmHg or diastolic blood pressure >95 mmHg). Systolic blood pressure rose normally during exercise but diastolic pressure was elevated (>95 mmHg) in 8 patients. Individual values for the cardiorespiratory and metabolic parameters can be found in Table 3.3.

TABLE 3.2. CARDIORESPIRATORY AND METABOLIC PARAMETERS AT REST AND DURING PEAK EXERCISE IN 10 PATIENTS WITH END-STAGE RENAL FAILURE

	RESTING	PEAK
EXERCISE		
Heart rate (beats.min^{-1})	98 ± 21	168 ± 12
Blood pressure (mmHg)		
Systolic	135 ± 11*	173 ± 18
Diastolic	89 ± 8*	107 ± 9
Oxygen consumption (ml O_2.kg^{-1}.min^{-1})	-	17.7 ± 3.6
Oxygen pulse (ml.O_2^{-1}.beat^{-1})	-	5.8 ± 1.4
Blood lactate concentration (mmol.l^{-1})	1.1 ± 0.4	3.4 ± 0.9
RER (units)	0.9 ± 0.1	1.1 ± 0.1
Ventilation (l.min^{-1})	12.2 ± 1.9	37.3 ± 14.6
Maximum workrate (Watts)	-	70 ± 25
Exercise duration (mins)	-	11 ± 3

ABBREVIATIONS: RER=respiratory exchange ratio.

Values are expressed as Mean ± SD for n = 10 except * n = 9

Table 3.3. INDIVIDUAL CARDIORESPIRATORY; METABOLIC AND ISOKINETIC MUSCLE STRENGTH PARAMETERS AT REST AND DURING PEAK EXERCISE IN 10 PATIENTS WITH END-STAGE RENAL FAILURE

	1	2	3	4	5	6	7	8	9	10
Heart rate	85 153	140 180	91 145	110 169	60 186	105 168	115 200	109 160	85 170	95 180
SBP	120 150	140 180	120 150	140 180	150 210	140 180	160 180	145 210	160 170	120 180
DBP	90 105	80 120	90 90	80 110	85 110	100 115	120 110	95 110	100 110	80 110
VO_2	17.2	13.7	14.2	17.7	21.3	18.5	19.0	13.7	25.3	16.8
Lactate	0.71 1.62	0.99 3.39	1.86 3.44	1.39 2.55	1.17 4.29	1.29 2.41	0.36 4.26	1.46 2.78	0.72 4.55	0.77 2.82
RER	1.00	1.12	1.12	1.27	1.25	1.10	1.16	0.98	1.20	1.10
Vent	9.5 24.8	11.5 25.7	11 25.9	13.7 53.1	13.4 54.3	12.7 36.5	13.0 51.9	9.5 14.6	* 56.5	15.7 29.4
Workrate	60	60	60	75	105	60	105	30	105	45
Duration	10	10	10	11	16	10	15	6	16	8
70 rpm	756	656	870	914	1228	813	1000	571	1129	756
90 rpm	770	787	970	1081	1410	823	1134	605	1319	750
110 rpm	850	760	1051	1252	1454	825	1276	626	1342	716
60 $^0.sec^{-1}$	59	69	75	74	120	73	87	59	98	55
90 $^0.sec^{-1}$	55	57	66	76	106	65	76	51	89	48
Hb	8.3	8.2	7.5	7.5	7.8	8.6	9.1	4.6	8.6	11.0
Hct	26	24	23	21	23	26	26	12	25	30

KEY: 1-10=individual patients (3,4,9=males);Heart rate ($beats.min^{-1}$); SBP=Systolic Blood Pressure (mmHg); DBP=Diastolic Blood Pressure (mmHg); VO_2=oxygen consumption ($ml.O_2\ kg^{-1}.min^{-1}$); Lactate=blood lactate concentration ($mmol.l^{-1}$); RER=Respiratory Exchange Ratio; Vent=Ventilation ($l.min^{-1}$); Workrate=maximal workrate achieved (Watts); Duration=Exercise duration (minutes); 70, 90, 110= Speeds tested on isokinetic cycle (Watts); 60, 90=Speeds tested on Cybex dynamometer (ft.lbs); Hb=haemoglobin ($g.dl^{-1}$); Hct=haematocrit (%).; *=missing value.

NOTE: Were 2 numbers appear together in a single cell the top number refers to resting value whereas the bottom number denotes the peak recorded value.

The peak oxygen consumption (17.7 ± 3.6 ml $O_2.kg^{-1}.min^{-1}$) was achieved at low peak rates of ventilation (37.3 ± 14.6 $l.min^{-1}$), at low peak blood lactate concentrations (3.4 ± 0.9 $mmol.l^{-1}$) and at very low peak workloads (70 ± 25 Watts), but at elevated peak respiratory exchange ratios of 1.1 ± 0.1.

Figure 3.1 shows the changes in oxygen consumption, heart rate, ventilation and blood lactate concentrations measured during the progressive exercise test. The linear and curve-fitting equations which describe the data are depicted on the Figure. The data are for all 10 patients for the first 6 minutes, for 9-7 patients up to 10 minutes, and for 3 patients up to 15 minutes. Data at 16 minutes are for 2 patients.

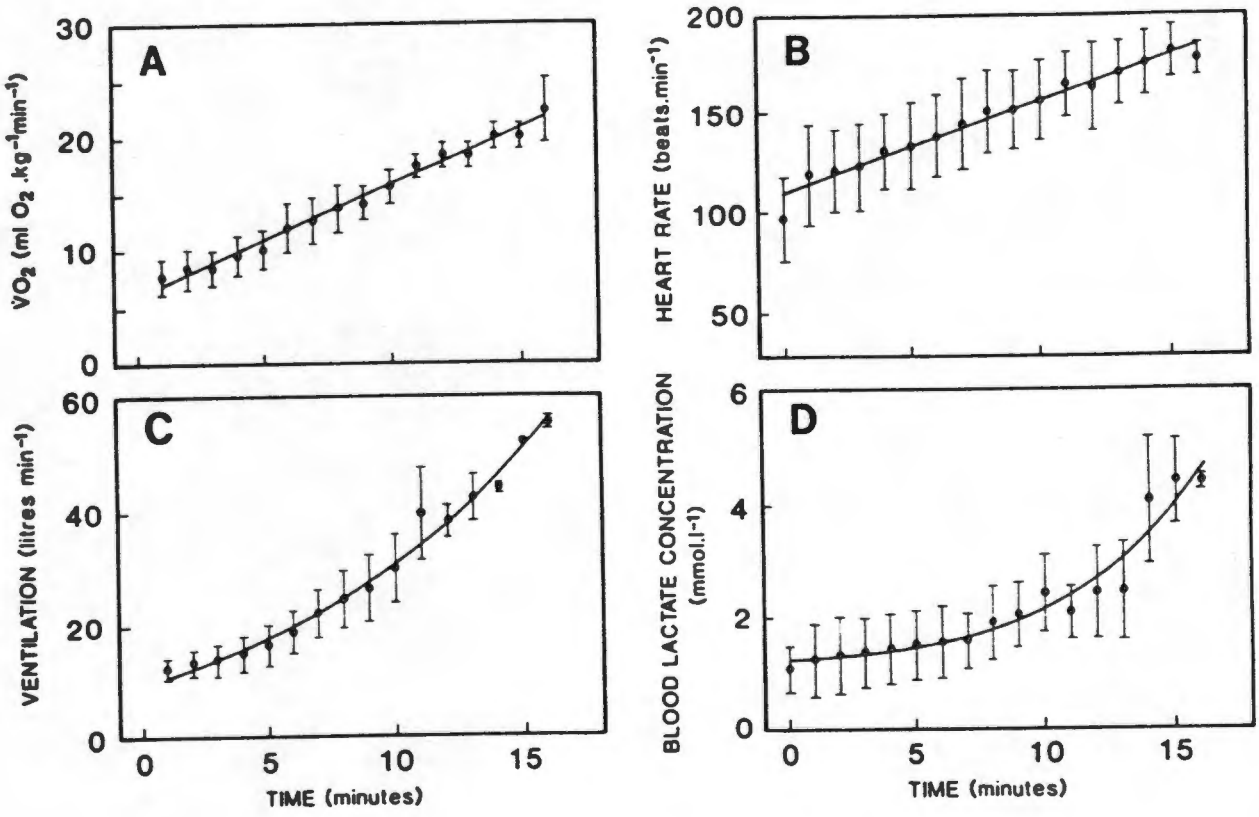

Figure 3.1: Changes in oxygen consumption, heart rate, ventilation and blood lactate concentrations during progressive exercise to exhaustion in 10 patients with end-stage renal failure. Note the absence of a plateau in oxygen consumption with increasing workrate (exercise duration).

Oxygen consumption rose linearly with increasing exercise duration (r^2 = 0.99) and in none of the patients did a

plateau in oxygen consumption develop [Noakes, 1988]. Blood lactate concentrations rose as an exponential function of exercise duration as previously described [Hughson et al., 1981; Dennis et al., 1991]. Ventilation also increased as an exponential function of exercise duration (r^2 = 0.99). All patients terminated exercise with blood lactate concentrations below 4 $mmol.l^{-1}$. Reasons for terminating the test include leg fatigue (n = 4), general fatigue (n = 3), dry mouth (n = 2) and thigh cramps (n = 1).

Peak isokinetic power measured on the cycle at 70,90 and 110 $r.min^{-1}$ was 869 ± 205, 965 ± 265 and 1015 ± 297 Watts respectively. Peak isokinetic torque measured using the Cybex dynamometer at 60 and 90 $degrees.sec^{-1}$ was 128 ± 27 and 117 ± 28 ft.lbs respectively. The changes in isokinetic strength with increasing crank or angular velocity was not significant ($p>0.05$).

Correlation analyses were performed between peak oxygen consumption ($mlO_2.kg^{-1}.min^{-1}$) and variables relating either to oxygen carrying capacity and transport (haemoglobin concentration,haematocrit; peak heart rate, respectively) or to isokinetic muscle strength. Table 3.4 shows that there was no significant correlation between peak oxygen consumption and variables relating to blood oxygen carrying capacity. However a significant correlation was found between peak oxygen consumption and isokinetic muscle strength measured with either the isokinetic cycle ergometer

($r = 0.73$ to $r = 0.84$; $p < 0.05$ to $p < 0.01$) or the Cybex Isokinetic Dynamometer ($r = 0.66$ to 0.68; $p < 0.05$).

TABLE 3.4 CORRELATION COEFFICIENTS FOR PEAK OXYGEN CONSUMPTION AND VARIABLES RELATING TO EITHER ISOKINETIC SKELETAL MUSCLE FUNCTION OR BLOOD OXYGEN CARRYING CAPACITY AND TRANSPORT

Variable	R value	p value
Peak isokinetic power		
Isokinetic cycle (Watts)		
at 70 $r.min^{-1}$	0.84	<0.01
at 90 $r.min^{-1}$	0.79	<0.01
at 110 $r.min^{-1}$	0.73	<0.05
Cybex dynamometer (ft.lbs)		
at $60^{o}.sec^{-1}$	0.66	<0.05
at $90^{o}.sec^{-1}$	0.68	<0.05
Peak lactate concentration ($mmol.l^{-1}$)	0.52	NS
Blood Oxygen Carrying Capacity		
Haematocrit (%)	0.35	NS
Haemoglobin concentration ($g.dl^{-1}$)	0.33	NS
Blood Oxygen Transport		
Peak Heart Rate	0.34	NS

NS = not significant

DISCUSSION

The possibility that muscle weakness may explain the poor exercise tolerance of patients with end-stage renal failure receiving chronic haemodialysis has been suggested by previous authors [Painter et al., 1986(b); Lundin et al., 1987], but direct evidence for this proposal has been lacking.

The first important finding of this study was to show that the patients terminated exercise with low peak heart rates and low peak ventilation rates, at low peak blood lactate concentrations and with low peak values for oxygen consumption (Figure 3.1). Furthermore a plateau in oxygen consumption was not identified in any patient [Noakes, 1988]. Had the anaemia been a significant component of the impaired exercise tolerance of renal haemodialysis patients, one might have expected higher, that is near maximal values for peak ventilation, peak heart rate and peak blood lactate concentrations [Lundin et al., 1987]. The low peak blood lactate concentrations as also measured by others [Barnea et al., 1980; Lundin et al., 1987] argue against the early onset of "anaerobic" metabolism as a cause of fatigue in these patients [Lundin et al., 1987]. It needs to be emphasized that the haematocrit and haemoglobin concentrations as well as the peak oxygen consumptions and peak achieved workloads measured in this study are similar

to those reported by others [Painter et al., 1986(b); Shalom et al., 1984; Roseler et al., 1980].

More convincing evidence that the anaemia could not explain the impaired exercise tolerance of these patients, was the failure to show any significant correlation between variables relating to oxygen carrying capacity or transport(haematocrit and haemoglobin concentration; peak heart rate, respectively) and either oxygen consumption or peak achieved workrate (Table 3.4). However, due to the small sample size statistical significance may be present but not observed. In contrast, there was a strong correlation between peak isokinetic muscle strength measured with either the isokinetic bicycle or the Cybex Isokinetic Dynamometer and peak oxygen consumption, exercise duration, peak ventilation and peak blood lactate concentrations.

These findings therefore confirm the suggestion made by Robertson et al. (1990), that muscle strength contributes significantly to the poor exercise tolerance in patients receiving chronic haemodialysis. Interestingly, the recent study of Lipkin et al. (1988), also found a significant correlation between isokinetic muscle power and peak oxygen consumption in patients with congestive heart failure. The correlation coefficient ($r = 0.86$) was similar to that found in this study ($r = 0.84$). Furthermore the correlations found in this study were almost identical to those reported by Robertson et al. (1990), viz. ($r = 0.68$ vs $r = 0.64$),

when VO_2 peak was correlated with isokinetic quadriceps muscle strength.

SUMMARY

Patients with end-stage renal failure receiving chronic haemodialysis have impaired exercise tolerance.

To distinguish between a central cardiorespiratory and a peripheral skeletal muscular origin for this fatigue, we measured exercise performance and peak oxygen consumption during a maximum exercise test in 10 patients receiving chronic haemodialysis. Skeletal muscle function was measured with an isokinetic cycle ergometer and a Cybex II isokinetic dynamometer.

Peak rates of oxygen consumption (17.7 ± 3.6 (Mean ±SD) ml $O_2.kg^{-1}.min^{-1}$), blood lactate concentrations (3.4 ± 0.9 $mmol.L^{-1}$), peak heart rates (168 ± 12 $beats.min^{-1}$) and rates of ventilation (37.3 ± 14.6 $L.min^{-1}$) were low but respiratory exchange ratios (1.1 ± 0.1) were compatible with maximal effort.

There was a significant correlation between isokinetic muscle strength and VO_2 peak, exercise duration, peak ventilation and peak blood lactate concentrations but not between haemoglobin concentration or hematocrit and these variables.

Therefore in renal haemodialysis patients, muscle contractile function may be one of several important contributing factors, present in renal failure, responsible

for the low exercise tolerance levels seens in these patients.

CHAPTER 4

ENHANCED SKELETAL MUSCLE FUNCTION IN PATIENTS WITH END-STAGE RENAL FAILURE RECEIVING RECOMBINANT HUMAN ERYTHROPOIETIN

INTRODUCTION

A number of recent studies have shown that recombinant human erythropoietin (EPO), which partially corrects the anaemia in patients with end-stage renal failure receiving chronic haemodialysis, also increases their capacity for maximal exercise [Robertson et al., 1990; Mayer et al., 1988; Mayer et al 1989; MacDougall et al., 1990; Bocker et al., 1988; Canadian Erythropoietin Study Group, 1990; Graf et al., 1987; Lundin et al., 1991]. Since $VO_{2\ peak}$ also increased significantly after EPO treatment in these studies, some authors have concluded that EPO increases the exercise tolerance of these patients by increasing their capacity for oxygen delivery to the active muscles during maximal exercise [Mayer et al., 1988; Mayer et al, 1989; Graf et al., 1987]. Hence, they interpret these findings as evidence for a central (cardiovascular) limitation for exercise tolerance in these patients.

However if, as suggested in Chapter 3, skeletal muscle oxygen deficiency does not limit the exercise tolerance of these patients even when they are anaemic, then an increased potential for oxygen delivery to the active muscles following correction of the anaemia, might not solely explain why EPO treatment increases the exercise tolerance of these patients.

Accordingly, in this study, we have performed maximal exercise tests before and after 3 months of EPO therapy, in a group of patients with end-stage renal failure receiving chronic haemodialysis. We wished to determine whether EPO therapy alters physiological variables during submaximal and maximal exercise. In addition, we studied the effect of EPO therapy on skeletal muscle contractile function measured with an isokinetic dynamometer.

Changes in these variables were compared with a control group of patients with end-stage renal failure who did not receive EPO therapy during the same experimental period.

MATERIALS AND METHODS

Nine adult patients receiving chronic haemodialysis volunteered for this study, which had been approved by the Ethics and Research Committee of the Faculty of Medicine of the University of Cape Town. Each patient underwent a full medical examination conducted by a renal physician and signed an informed consent form prior to testing. All were on stable medical regimens, diets and haemodialysis programmes involving a 12 $hr.week^{-1}$ haemodialysis regimen. Fresenius A 2008 C haemodialysis machines (Fresenius, Germany) were used, with the haemodialysis concentrate containing acetate. PCR (Protein Clearance Ratio) and $KT.V^{-1}$ (K = constant; T = duration of haemodialysis; and V = blood volume of patient) values were not yet being used to assess adequacy of dialysis, however the clinical condition

of the patients were used to monitor their dialysis regimens. Furthermore, the haemodialysis regimens of the patients who received EPO did not change appreciably during or after the study.

At the time of doing the study EPO had been introduced into South Africa only on a trial basis and this was one of the first studies to use EPO in this country. Only sufficient EPO was made available for 5 patients. One of the patients put onto EPO did not wish to be part of our study. Therefore our experimental group was limited to 4 patients.

All testing was done the day after patients had received their last haemodialysis treatment for the week. The initial test was performed prior to commencement of EPO treatment and the second test three months later. The control group received normal medical care during this 3 month period but were not given EPO.

Selection to the experimental or control groups was made purely on medical grounds by the renal physicians caring for the patients and was not influenced by the requirements of this study. The lower initial haemoglobin concentrations of the group who received EPO therapy is an expected consequence of this selection.

The methods used to determine haemoglobin concentration, peak oxygen consumption, exercising blood lactate

concentrations and isokinetic muscle strength were the same as those described in the study reported in Chapter 3.

In brief, haemoglobin concentration was determined the day before the patients underwent the maximal exercise test. The next day the patients reported to the exercise laboratory for measurement of peak oxygen consumption using an electronically calibrated cycle ergometer. The exercise test involved continuous progressive increments in workrate until the patient voluntarily terminated the test. The test started at 0 Watts and increased by 15 Watts every 2 minutes. Samples for measurement of blood lactate concentration were collected at rest and 30 seconds before the end of each workrate.

All nine patients completed the maximum exercise test; one patient commenced EPO treatment before the initial muscle strength testing could be scheduled. Blood lactate concentrations during the maximum exercise test were not analyzed in one patient due to inadequate blood samples being collected during the test.

During the week following the maximal exercise test, patients performed maximal isokinetic exercise on the Cybex Isokinetic Dynamometer (Lumex Inc., Ronkonkoma, NY) for determination of peak quadriceps isokinetic torque development at limb contraction velocities of 45, 60, 90 and 180 degrees.second^{-1}. The data were reduced and analyzed as previously described in Chapter 3.

Statistical methods

Group data for each variable are expressed as mean ±SD. A one-sided paired sample Students' t-test was used to compare values at the start and termination of the study. Data for blood lactate concentrations during exercise were fitted with the non-linear regression programme of the Institute for Scientific Information Graph Pad (Philadelphia, PA). Straight lines were fitted by conventional least squares analyses for the oxygen consumption data. Statistical significance was established at the level of $p < 0.025$ because of the small sample size.

RESULTS

The personal characteristics of the patients are listed in Table 4.1. That the division into control and experimental groups occurred according to gender was beyond the control of the experimenters and is justified by the higher haemoglobin concentrations of the control patients (Table 4.2). However there is no evidence that gender influences the physiological responses to exercise or the adaptations to training in these patients [Kempeneers et al., 1990].

Only one patient (Case 4 in the experimental group) was on anti-hypertensive medication; initially the treatment was metoprolol 100 mg per day but this was changed to verapamil 120 mg tds after EPO therapy had been started. The extent

to which this change in medication effected our results was unfortunately impossible to assess.

None of the measured variables changed in the control group during the 3 month period (Table 4.2 and 4.3). Samples from the control group for blood lactate analysis in the follow-up study were inadvertently discarded before analysis could be performed.

In contrast to the control group, haemoglobin concentrations were significantly increased ($p < 0.01$) following the 3 months of EPO therapy whereas serum ferritin concentrations did not change (Table 4.4 and 4.5). Peak achieved workrate ($p < 0.025$), exercise duration ($p < 0.025$), peak oxygen consumption ($p < 0.025$) and peak ventilation ($p < 0.025$) all increased significantly during the maximum exercise test after EPO treatment (Table 4.4 and 4.5). Resting heart rate decreased with therapy but peak heart rate, resting and peak systolic and diastolic blood pressures, peak and resting blood lactate concentrations and peak respiratory exchange ratios (RER) were not altered (Table 4.4 and 4.5). All patients complained that leg fatigue was the predominant symptom terminating exercise.

TABLE 4.1. PERSONAL CHARACTERISTICS AND CAUSES OF RENAL FAILURE IN NINE PATIENTS WITH END-STAGE RENAL FAILURE RECEIVING CHRONIC HAEMODIALYSIS

PATIENT	SEX	AGE (YR)	WEIGHT (KG)	CAUSE OF FAILURE	DURATION ON DIALYSIS (YR)
(1) EXPERIMENTAL GROUP					
1	M	41	58	CGN	2
2	M	47	64	CGN	4
3	M	48	48	CGN	6
4	M	18	44	BCN	6
Mean		38.5	53.3		4.5
(± SD)		(14)	(9)		(2)
(2) CONTROL GROUP					
5	F	55	57	HT	4
6	F	26	62	CGN	13
7	F	22	48	RN	8
8	F	47	57	PKD	2
9	F	43	58	CGN	3
Mean		38.6	56.4		6.0
(± SD)		(14)	(5.1)		(4.5)

KEY: Abbreviations CGN = Chronic Glomerulonephritis; BCN = Bilateral Cortical Necrosis; HT = Hypertensive renal disease; PKD = Polycystic kidney disease; RN = Reflux nephritis.

TABLE 4.2. HAEMATOLOGICAL, MAXIMAL EXERCISE AND ISOKINETIC MUSCLE TEST RESULTS BEFORE AND AFTER EPO TREATMENT IN 5 CONTROL PATIENTS WITH CHRONIC RENAL FAILURE NOT RECEIVING EPO TREATMENT DURING A 3 MONTH PERIOD

	Initial	Three months later
Haemoglobin ($g.dL^{-1}$)	7.9 ± 0.9	7.0 ± 1.4
Maximal Exercise Test:		
Peak achieved workrate (Watts)	70 ± 20	66 ± 13
Duration (mins)	10.8 ± 2.4	10.2 ± 2.2
Peak VO_2 (ml $O_2.kg^{-1}.min^{-1}$)	15.8 ± 2.2	15.2 ± 1.2
Peak ventilation ($L.min^{-1}$)	33.0 ± 11.6	38.7 ± 14.9
Peak RER	1.14 ± 0.1	1.17 ± 0.1
Heart rate: Rest ($b.min^{-1}$)	110 ± 21	106 ± 21
Max	170 ± 22	158 ± 17
Systolic B P: Rest (mmHg)	132 ± 19	132 ± 24
Max	158 ± 22	164 ± 29
Lactate: Rest ($mmol.L^{-1}$)	0.97 ± 0.55	-
Max	3.0 ± 1.1	-
Isokinetic Muscle Function: Peak torque at (foot pounds) at:		
$45^{o}.sec^{-1}$	76 ± 15	79 ± 17
$60^{o}.sec^{-1}$	69 ± 12	76 ± 15
$90^{o}.sec^{-1}$	61 ± 10	61 ± 10
$180^{o}.sec^{-1}$	46 ± 7	50 ± 6

KEY: Abbreviations EPO = Recombinant Human Erythropoietin; VO_2 = Oxygen Consumption; B P = Blood Pressure.

TABLE 4.3. INDIVIDUAL HAEMATOLOGICAL, MAXIMAL EXERCISE AND ISOKINETIC MUSCLE TEST RESULTS BEFORE AND AFTER EPO TREATMENT IN 5 CONTROL PATIENTS WITH CHRONIC RENAL FAILURE NOT RECEIVING EPO TREATMENT DURING A 3 MONTH PERIOD

	1	2	3	4	5
Haemoglobin	8.8 8.1	9.1 6.3	6.6 5.0	7.5 8.2	8.3 7.7
Workrate	60 60	60 60	105 90	60 60	60 60
VO_2	13.7 15.7	14.8 15.9	19 16.5	14.2 14.6	17.2 13.4
Duration	10 10	9 9	15 14	10 9	10 9
Ventilation	25.7 28.6	36.7 39.7	51 62.6	25.9 38.6	24.8 24
RER	1.12 1.15	1.28 1.19	1.16 1.32	1.12 1.15	1.00 1.06
Resting Heart rate	140 135	90 88	115 123	91 90	113 96
Peak Heart rate	180 170	174 165	200 168	145 160	153 128
Rest SBP	140 140	110 120	160 170	120 110	130 120
Peak SBP	180 200	130 150	180 190	150 140	150 140
Rest Lactate	0.99 *	0.92 *	0.36 *	1.86 *	0.71 *
Peak Lactate	3.39 *	2.08 *	4.26 *	3.44 *	1.62 *
45	74 88	79 75	96 103	78 73	54 58
60	69 86	69 67	87 95	67 72	52 59
90	57 76	61 53	76 83	61 69	48 55
180	50 56	38 40	54 54	49 52	40 48

KEY: 1-5=Individual patients; Haemoglobin ($g.dl^{-1}$); Workrate=Peak workrate(Watts); VO2=Peak oxygen consumption ($mlO_2.kg^{-1}.min^{-1}$); Duration=exercise duration (minutes); Ventilation=peak ventilation ($l.min^{-1}$); RER=peak respiratory exchange ratio; Heart rate ($beats.min^{-1}$); SBP=Systolic blood pressure (mmHg); Lactate=blood lactate concentration ($mmol.l^{-1}$); 45,60,90,180=Speeds tested on Cybex isokinetic dynamometer ($degrees.sec^{-1}$); *=missing value

NOTE: The top number in each cell refers to the initial test and the bottom number refers to the test 3 months later

TABLE 4.4. GROUP HAEMATOLOGICAL, MAXIMAL EXERCISE AND ISOKINETIC MUSCLE TEST RESULTS BEFORE AND AFTER EPO TREATMENT IN 4 PATIENTS WITH CHRONIC RENAL FAILURE RECEIVING EPO THERAPY

	Before EPO therapy	After EPO therapy
Haemoglobin ($g.dL^{-1}$)	5.4 ± 0.8	9.9 ± 0.4*
Serum ferritin ($ng.mL^{-1}$)	2910 ± 4604	2538 ± 4267
Maximal Exercise Test:		
Peak achieved workrate (Watts)	79 ± 12	109 ± 19**
Duration (mins)	11.8 ± 1.6	16.0 ± 2.7**
Peak VO_2 (ml $O_2.kg^{-1}.min^{-1}$)	20.2 ± 5.8	27.3 ± 8.5**
Peak ventilation ($L.min^{-1}$)	36.8 ± 7.8	44.7 ± 9.1**
Peak RER	1.1 ± 0.05	1.1 ± 0.03
Heart rate: Rest ($b.min^{-1}$)	91 ± 16	69 ± 9
Max	161 ± 20	155 ± 12
Systolic B P: Rest (mmHg)	146 ± 29	133 ± 29
Max	173 ± 33	175 ± 45
Lactate: Rest ($mmol.L^{-1}$)	1.77 ± 0.34	0.95 ± 0.12
Max	3.46 ± 0.50	5.50 ± 1.4
Isokinetic Muscle Function: Peak torque (foot pounds) at:		
$45^{o}.sec^{-1}$	108 ± 9	125 ± 5*
$60^{o}.sec^{-1}$	105 ± 9	121 ± 6*
$90^{o}.sec^{-1}$	98 ± 5	114 ± 9*
$180^{o}.sec^{-1}$	65 ± 2	78 ± 2*

KEY: Abbreviations EPO = Recombinant Human Erythropoietin; VO_2 = Oxygen Consumption; B P = Blood Pressure.
Significance * $p < 0.01$; ** $p < 0.025$.

TABLE 4.5. INDIVIDUAL HAEMATOLOGICAL, MAXIMAL EXERCISE AND ISOKINETIC MUSCLE TEST RESULTS BEFORE AND AFTER EPO TREATMENT IN 4 PATIENTS WITH CHRONIC RENAL FAILURE RECEIVING EPO THERAPY

	1	2	3	4
Haemoglobin	5.3 10.8	5.0 9.8	4.6 9.3	6.7 9.9
Workrate	60 90	90 135	90 120	75 90
VO_2	19.4 24.0	29.8 41.2	17.3 26.6	14.5 17.9
Duration	9 13	13 20	13 17	12 14
Ventilation	34.7 37.1	50.1 60.2	31.2 42.2	30.8 39.4
RER	1.08 1.08	1.07 1.08	1.18 1.16	1.05 1.10
Resting Heart rate	90 79	115 58	88 62	70 75
Peak Heart rate	169 159	187 159	154 165	132 135
Rest SBP	120 90	190 160	120 120	155 160
Peak SBP	140 120	200 190	140 150	170 240
Rest Lactate	2.45 0.93	1.34 0.75	1.52 0.89	* *
Peak Lactate	3.43 4.76	4.35 8.21	2.62 3.61	* *
45	* *	106 111	89 108	110 120
60	* *	109 116	86 107	103 112
90	* *	99 108	80 96	95 110
180	* *	67 70	63 71	66 56

KEY: 1-4=Individual patients; Haemoglobin ($g.dl^{-1}$); Workrate=Peak workrate(Watts); VO2=Peak oxygen consumption ($ml O_2.kg^{-1}.min^{-1}$); Duration=exercise duration (minutes); Ventilation=peak ventilation ($l.min^{-1}$); RER=peak respiratory exchange ratio; Heart rate ($beats.min^{-1}$); SBP=Systolic blood pressure (mmHg); Lactate=blood lactate concentration ($mmol.l^{-1}$); 45,60,90,180=Speeds tested on Cybex isokinetic dynamometer ($degrees.sec^{-1}$); *=missing value
NOTE: The top number in each cell refers to the initial test and the bottom number refers to the test 3 months later

Figure 4.1 shows changes in oxygen consumption (A), heart rate (B), ventilation (C) and blood lactate concentrations (D) with increasing workrate before and after treatment in the experimental patients.

Before EPO therapy, one patient reached 60 Watts, one 75 Watts and two, 90 Watts. After treatment two patients reached 90 Watts, one 120 Watts and one, 135 Watts. As a result of the higher maximal workloads achieved by all patients, the peak oxygen consumption increased by 26 $\pm$ 5%. None of the patients demonstrated a `plateau' [Kempeneers et al, 1990; Noakes TD, 1988] in peak oxygen consumption, before or after EPO treatment. In addition, oxygen consumption at all submaximal workloads (0 - 75 Watts) were essentially identical in both tests (Figure 4.1).

Heart rate increased linearly with increasing workrate in experimental patients both before and after EPO therapy and was reduced by approximately 20 beats.min^{-1} at all workloads (Figure 4.1, line B).

Ventilation volume increased as a curvilinear function of work rate and was marginally lower at each workrate after EPO treatment (Figure 4.1, line C).

Blood lactate concentrations also rose as a curvilinear function of increasing workrate (Figure 4.1, line D). There was a trend for these concentrations to be reduced at rest

and during submaximal exercise and peak concentrations to be increased, but these differences failed to reach statistical significance.

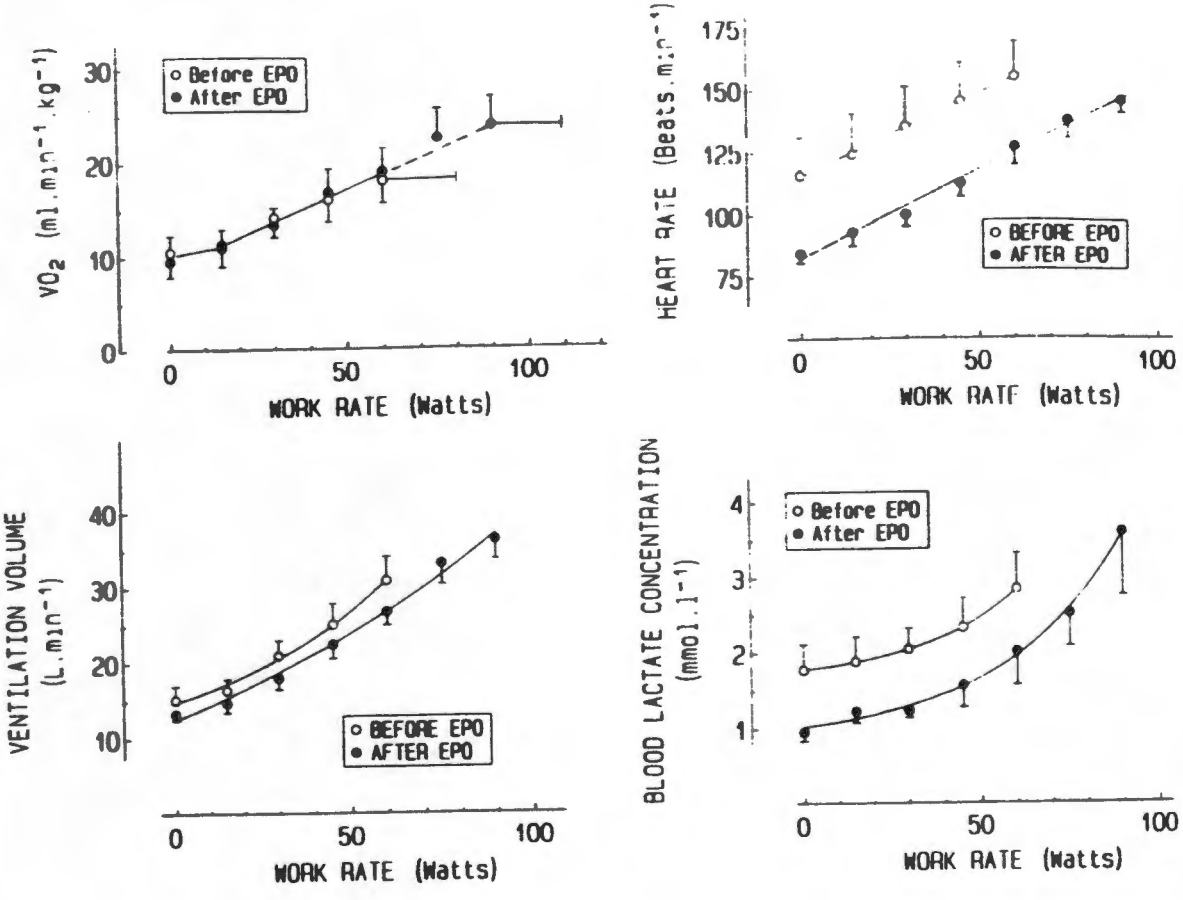

Figure 4.1: Changes in oxygen consumption (VO_2) (top left), heart rate (top right), ventilation volume (bottom left) and blood lactate concentrations (bottom right) during progressive exercise to exhaustion in 4 patients with end-stage renal failure before and after EPO therapy.

Peak isokinetic strength increased significantly ($p < 0.01$) at all testing velocities following EPO treatment (Table 4.3) but was unchanged in the control patients (Table 4.2). Patients in the experimental group had higher isokinetic

muscle strength and higher VO_2 peak before and, especially, after EPO therapy. We believe the initial higher values to be a gender-related difference.

TABLE 4.4. CORRELATION COEFFICIENTS FOR PEAK OXYGEN CONSUMPTION AND VARIABLES RELATING TO EITHER ISOKINETIC MUSCLE FUNCTION OR BLOOD OXYGEN CARRYING CAPACITY

Variable	r Value	P Value
Isokinetic Muscle Strength		
at 45 $^{o}.sec^{-1}$	0.54	<0.05
at 60 $^{o}.sec^{-1}$	0.64	<0.02
at 90 $^{o}.sec^{-1}$	0.62	<0.02
at 180 $^{o}.sec^{-1}$	0.67	<0.01
Blood Oxygen Carrying Capacity		
haemoglobin concentration	0.39	NS

KEY: ABBREVIATIONS $deg.sec^{-1}$ = degrees per second; NS = not significant.

As in the previous study [Chapter 3], there were significant correlations between isokinetic muscle strength and VO_2 peak, but not between haemoglobin content and VO_2 peak (Table 4.4). Figure 4.2 shows a significant linear regression ($r = 0.62$; $p < 0.02$) between VO_2 peak and peak torque as measured at 90 $^{o}.sec^{-1}$. The correlations and the

regression were performed on all patients, before and after the 3 month trial period.

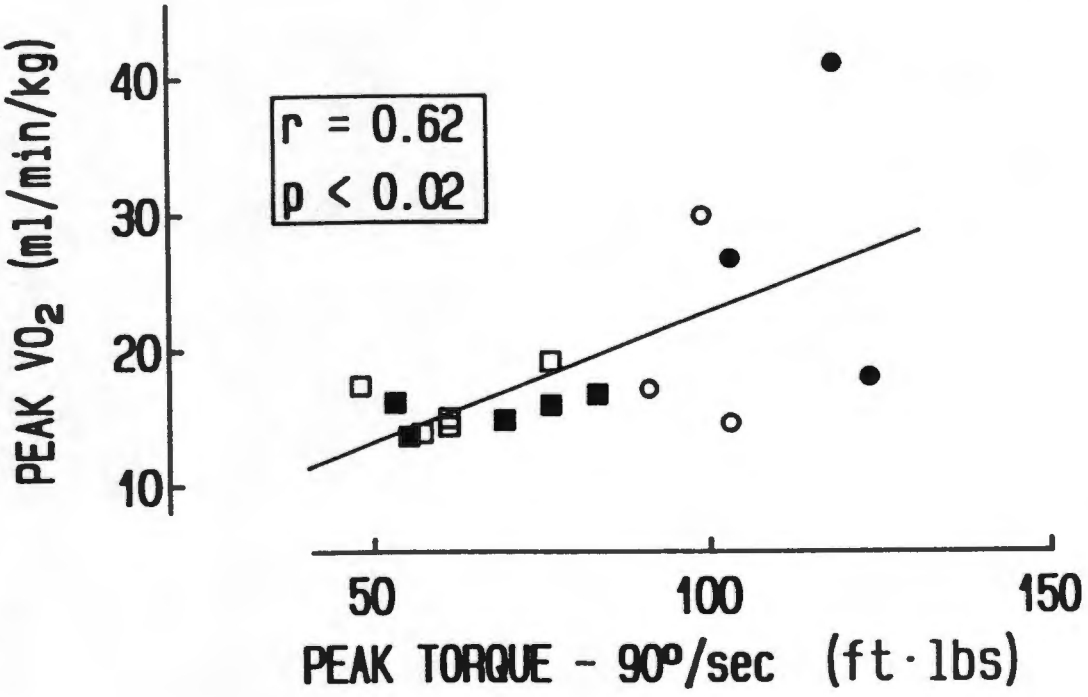

Figure 4.2: Relationship between peak oxygen consumption (VO_2 peak) and peak torque at 90 $^{o}.sec^{-1}$ using both groups results, before and after the 3 month period. The open and closed circles are for the EPO group, before and after treatment, respectively. The open and closed squares are for the control group, before and after the 3 month period, respectively.

Although not specifically measured, all patients in the experimental group indicated that since starting therapy, they coped better with activities of daily living such as walking and stair climbing.

DISCUSSION

Partial correction with EPO therapy of the anaemia experienced by patients with end-stage renal failure has been shown to increase their exercise tolerance (Robertson et al., 1990; Lundin et al., 1991]. It would seem logical to presume that this results from enhanced oxygen delivery to the exercising muscles [Mayer et al., 1988; Mayer et al., 1989; Graf et al., 1987].

However Robertson et al. (1990) concluded that the increased exercise tolerance resulting from EPO therapy was less than might be expected from an equivalent elevation of haemoglobin concentrations in patients with anaemia of other causes. They concluded that: "These comparisons suggest that some as yet unidentified factor besides transport of oxygen to tissues is a major determinant of the maximal exercise performance of the haemodialysis patients.". As those authors, like us, found a significant correlation ($r = 0.64$) between isometric muscle strength and peak oxygen consumption, they concluded that muscle weakness may be responsible for the low $VO_{2\ peak}$ measurements and poor exercise tolerance of haemodialysis patients even after partial correction of their anaemia. Support for this interpretation comes from a number of other studies and is confirmed by the findings of the current investigation.

As early as 1987, Lundin et al. (1987) observed that patients with end-stage renal failure terminated maximal

exercise because of leg fatigue and with only small changes from base-line values for arterial pH and lactate concentrations. They concluded that "early, unexplained muscle fatigue" which was not due to "muscle hypoxia" caused these patients to terminate exercise well before "estimated maximum cardiac output and muscle 0_2 levels" had been reached. In their most recent study, these workers [Lundin et al., 1991] reported that there was no correlation between the increase in circulating haemoglobin concentration and the VO_2 peak following EPO therapy. Metra et al. (1991), have also reported the same observation.

Subsequently essentially the same findings in both renal transplant recipients who were not anaemic [Kempeneers et al., 1990] were reported from this laboratory. Furthermore, in Chapter 3, anaemic patients with end-stage renal failure showed the same response. In patients from both these groups, peak exercise terminated without evidence for a central cardiovascular limitation to exercise [Noakes, 1988] which might have been shown by a `plateau' in oxygen consumption with increasing workrate, near maximal heart rates and rates of ventilation, as well as markedly elevated peak blood lactate concentrations.

Furthermore, as confirmed by the findings of Robertson et al. (1990), we also found that isokinetic muscle strength predicted peak oxygen consumption in anaemic patients with chronic renal failure receiving chronic haemodialysis [Chapter 3].

Thus, like Robertson et al. (1990), we have concluded that muscle strength probably limits the exercise tolerance of renal patients both before [Chapter 3] and after renal transplantation [Kempeneers et al., 1990] and that the important effect of exercise training, at least in renal transplant recipients, may be to increase muscle strength [Kempeneers et al., 1990].

The findings of this study are therefore in agreement with these conclusions. For the patients in this study also terminated exercise, both before and after EPO treatment, with low heart rates, low rates of ventilation and low blood lactate concentrations (Table 4.3) and without evidence for a `plateau' in oxygen consumption (Figure 4.1). Furthermore, oxygen consumption at submaximal workloads was unaltered by EPO therapy (Figure 4.1). Blood lactate concentrations were reduced both at rest and during submaximal exercise (Figure 4.2), but this effect was not statistically significant. These findings mirror those reported by Robertson et al. (1990).

Thus the ability of the patients to continue exercising at those workloads at which they had terminated exercise prior to EPO treatment, could not have been due to enhanced oxygen delivery to the active muscles after EPO therapy as absolute oxygen consumption at that workrate had not changed.

Accordingly, we conclude that the reason why EPO therapy enhanced the exercise tolerance of these patients was not simply a result of increased haemoglobin content causing enhanced oxygen delivery to the active muscle during exercise. Moreover, we suggest that associated with the increase in haemoglobin concentration was a significant improvement in muscle strength which allowed the patients to tolerate higher workloads. Consequently by achieving higher peak workloads their $VO_{2\ peak}$ increased simultaneously.

There are three possible explanations for the increased isokinetic muscle strength measured in these patients after EPO therapy.

First, the increased levels of habitual daily activity reported by all patients after EPO treatment may have produced a muscle training effect. This does not, however, explain why EPO therapy should have increased the levels of habitual physical activity in these patients in the first place.

Alternatively, the anaemia may cause a regulated alteration in skeletal muscle contractile function by mechanisms other than a reduction in oxygen delivery to the muscles. An analogous situation may occur in cardiac patients in whom skeletal muscle dysfunction may occur and is probably the factor limiting their exercise tolerance [Lipkin et al., 1988; Massie et al., 1987(a); Massie et al., 1987(b)]. Possibly chronic anaemia also impairs skeletal muscle

function which recovers only when the anaemia is wholly or partially corrected.

However this cannot be the complete answer because some researchers have been unable to find significant correlations between changes in exercise tolerance in anaemic patients with end-stage renal failure following acute changes in haemoglobin concentrations following blood transfusions [Sill et al., 1972] or renal transplantation [Painter et al., 1986(a)].

Paradoxically, those studies which show that changes in total haemoglobin content predict changes in exercise tolerance in renal patients [Mayer et al., 1988; Mayer et al., 1989; Clyne et al., 1989] are also compatible with the postulate that anaemia causes impaired muscle contractile function. Thus they cannot be interpreted simply as evidence that the correction of the anaemia acts exclusively by reducing skeletal muscle hypoxia during exercise.

A third factor which may be influenced by EPO therapy and which may contribute to the low exercise tolerance of these patients, is the chronic acidosis characteristic of end-stage renal failure. Sutton et al. (1981) found that experimentally-induced metabolic acidosis significantly reduces peak blood lactate concentrations and exercise performance during maximal exercise. Metabolic acidosis has also been shown to increase muscle catabolism [Mitch et al., 1989]. Possibly the metabolic acidosis associated with

chronic renal failure may also be partially responsible for the chronically-impaired skeletal muscle function in these patients.

Unfortunately we did not study the effect of EPO therapy on the acid-base balance of these patients during exercise. We did, however, note a trend for blood lactate concentrations to be lower after EPO therapy (Figure 4.1) suggesting that acid-base status may well have been influenced, possibly by the increased buffering capacity of the larger red cell mass after EPO therapy.

In summary, this study confirms that the exercise tolerance of patients with end-stage renal failure increases following partial correction of their anaemia with EPO therapy [Robertson et al., 1990; Mayer et al., 1989; MacDougall et al., 1990; Bocker et al., 1988; Canadian Erythropoietin Study Group, 1990; Graf et al., 1987; Lundin et al., 1991]. However we show that the mechanism for this effect may not be that EPO simply enhances oxygen delivery to the active muscles during exercise by increasing the circulating haemoglobin concentration.

Rather we confirm the findings of Robertson et al., 1990 that EPO therapy is associated with enhanced skeletal muscle function in these patients. This could result either from increased levels of daily physical activity in these patients following partial correction of their anaemia; or possibly because EPO therapy in some ways enhances the

impaired skeletal muscle contractile function that results either from the primary disease or from the associated anaemia. One possible way in which EPO therapy may enhance skeletal muscle function is the proposed anabolic effect [Zehnter et al., 1988] supported by findings of increased muscle protein content during one year's treatment with EPO [Barany et al., 1991]. Unfortunately dietary protein intake and the effects of EPO on those intakes were not measured in the present study. Future studies should include dietary evaluations. The finding that circulating haemoglobin concentration is not related to either $VO_{2\ peak}$ or skeletal isokinetic muscle strength raises questions about its value as the single measure on which to base decisions regarding the necessity of starting EPO therapy. Perhaps a measure of exercise tolerance should be included in this evaluation.

Summary

The mechanisms by which treatment with EPO enhances the exercise tolerance of patients with end-stage renal failure receiving chronic haemodialysis is not known.

We therefore compared changes in exercise tolerance and peak oxygen consumption during a maximum exercise test, and isokinetic skeletal muscle function in 4 experimental patients and 5 controls immediately before, and after 3 months during which the experimental group received EPO.

Haemoglobin concentrations rose significantly with treatment (5.4 ± 0.8 vs 9.9 ± 0.4 [mean ±SD] $g.dL^{-1}$; $p < 0.02$) as did peak rates of oxygen consumption (20.2 ± 5.8 vs 27.3 ± 8.5 ml $O_2.kg^{-1}.min^{-1}$; $p < 0.025$). Neither oxygen consumption during submaximal exercise nor blood lactate concentrations at exhaustion were influenced by EPO treatment, although blood lactate concentrations at rest and during submaximal exercise were reduced. Peak quadriceps isokinetic muscle strength at three different contraction velocities improved significantly ($p < 0.01$). None of these variables changed in the control group.

Identical rates of oxygen consumption during submaximal exercise both before and after treatment, low and unchanged peak blood lactate concentrations and the absence of a `plateau' in oxygen consumption during maximal exercise indicate that peripheral oxygen supply was not solely

responsible for limiting maximal exercise either before or after treatment with EPO. The observation that submaximal as well as maximal heart rates were lower following partial correction of their anaemia, does not necessarily indicate better peripheral oxygen blood flow. Rather, we suggest that stroke volume may have increased as a result of the correction of anaemia. Future investigations should measure variables such as stroke volume to test this hypothesis.

Therefore, the improved exercise tolerance of renal patients who receive EPO therapy does not appear to be solely due to reversal of muscle hypoxia which develops during maximal exercise but could result from an effect of EPO therapy on skeletal muscle contractile function. Confirmation of this hypothesis would require larger sample sizes; assessment of nutritional status, especially protein intake, before and after EPO therapy; direct measurement of muscular oxygen delivery and utilization during exercise; as well as changes in acid-base status.

CHAPTER 5

MORPHOLOGICAL FEATURES OF THE MYOPATHY ASSOCIATED WITH CHRONIC RENAL FAILURE

INTRODUCTION

As early as 1974, Floyd et al. (1974), showed that the skeletal muscles of renal patients receiving chronic haemodialysis showed significant electromyographic and morphological abnormalities but without evidence of neuropathic changes.

Our earlier studies found that skeletal muscle weakness is an important factor limiting the maximum exercise tolerance of patients who have either undergone renal transplantation [Kempeneers et al., 1990] or who are receiving chronic haemodialysis [Chapter 3] or therapy with erythropoietin (EPO) [Chapter 4]. Skeletal muscle biopsies performed in a group of physically-trained renal transplant recipients showed an unexpected predominance of Type II skeletal muscle fibres and a reduced skeletal muscle oxidative capacity (QO_2) [Kempeneers et al., 1990] but more detailed histological studies were not performed.

This chapter describes the histological findings in skeletal muscle biopsies of patients undergoing haemodialysis for end-stage renal failure. The exercise tolerance of these patients was shown to be limited by skeletal muscle weakness [Chapter 3].

MATERIALS AND METHODS

Eight of the ten patients receiving haemodialysis for end-stage renal failure studied in Chapter 3 consented to have skeletal muscle biopsies performed. Characteristics of these patients was their very low peak exercise tolerance measured as the peak oxygen consumption ($VO_{2\ peak}$). All patients were on a 12 hour per week dialysis programme and their dialysate contained acetate. The research protocol had been approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town.

A piece of muscle was removed from the vastus lateralis muscle under local anesthesia according to the open biopsy method described by Dubowitz et al. (1985). The biopsy fragment was divided into four sections: one piece for routine light microscopy was frozen in iso-pentane cooled in liquid nitrogen; the section for histology was cut on a standard cryostat freezing microtome; several fragments were fixed in buffered gluteraldehyde and prepared in the usual manner for transmission electron microscopy; the remainder was used for the measurement of muscle oxidative capacity (QO_2) using the technique described by Ivy et al. (1980), as modified in this laboratory [Lambert et al., 1989].

Blood samples were drawn routinely from these patients prior to their dialysis and analysed according to conventional techniques for haemoglobin concentration and haematocrit and

serum or plasma concentrations of total protein, albumin, urea, creatinine, calcium, inorganic phosphate and bicarbonate. Serum parathormone concentrations were determined by radio-immune assay using N-tact PTH IRMA (Incstar Corporation, Stillwater, Minnesota, 55082).

Control samples for muscle histological analysis were obtained from 7 athletic subjects undergoing arthroscopic knee surgery and from another 5 subjects who were untrained but otherwise healthy. Muscle biopsy samples were collected using a closed biopsy technique, but were prepared and analyzed using the identical techniques that were used for the patient samples.

Habitual dietary intake was calculated from a 2-day dietary record in which the weight of all foodstuffs ingested were reported. Dietary analysis was performed using the Floro Diet Data Programme (Durban, South Africa).

RESULTS

The physical characteristics of the patients are listed in Table 5.1. Patients had been receiving haemodialysis for a mean of 5 years; their renal failure had been caused by two diseases; glomerulonephritis and hypertensive renal disease.

Table 5.2 lists biochemical, haematological and nutritional data in the patients with chronic renal failure. Blood samples were collected immediately prior to dialysis; hence serum urea, creatinine and inorganic phosphate concentrations were elevated whereas the plasma bicarbonate concentration was reduced. Haematocrit, haemoglobin concentration and serum total protein and albumin concentrations were within the normal range. Serum parathormone concentrations were elevated above the normal range of 10-55 $pg.ml^{-1}$ in 6 of the 8 patients.

Dietary analysis in 7 patients showed a mean protein intake of 0.96 (± 0.17) $g.kg^{-1}.day^{-1}$; slightly less than the recommended intake of 1.2 $g.kg^{-1}.day^{-1}$. Mean daily energy intake of 27.8 (± 6.5) $Kcal.kg^{-1}.day^{-1}$ was also less than the recommended intake of 35 $Kcal.kg^{-1}.day^{-1}$. Mean dietary composition of 48 (± 7)% carbohydrate, 37 (± 8)% fat and 15 (± 2)% protein was within the expected range [Table 5.2].

Muscle oxidative capacity (QO_2) (19.5 ± 5.7 µl $O_2.g^{-1}.hr^{-1}$: Mean ± SD) was not different from values previously reported

in physically trained renal transplant recipients (21.3 ± 6.6 µl $O_2.g^{-1}.hr^{-1}$) [Kempeneers et al., 1990], but was significantly lower ($p < 0.05$) than values measured in a group of trained female endurance athletes (28.3 ± 7.1 µl $O_2.g^{-1}.hr^{-1}$).

TABLE 5.1. PATIENT CHARACTERISTICS

Sex	Age (Yrs)	Ht (m)	Wt (kg)	Duration of dialysis (Yrs)	Cause of renal failure	Serum PTH conc* ($pg.ml^{-1}$)
F	23	1.51	44.8	0.3	GN	550.7
F	37	1.55	45.1	7	HT	164.2
F	51	1.52	51.8	1	HT	101.0
M	26	1.64	62.2	5	GN	675.5
F	43	1.55	58.2	3	GN	340.6
F	55	1.59	57.0	4	HT	8.4
F	22	1.49	48.0	8	GN	172.1
F	26	1.64	62.1	13	GN	22.1
Mean (±SD)	34 (12)	1.56 (0.06)	53.7 (7.2)	5 (4)		254.0 (24.6)

Key: GN = glomerulonephritis
HT = hypertensive renal disease
PTH = parathyroid hormone
conc = concentration

* Normal range 10-55 $pg.ml^{-1}$

TABLE 5.2 PRE-DIALYSIS BIOCHEMICAL, HAEMATOLOGICAL AND NUTRITIONAL MEASUREMENTS IN 8 PATIENTS WITH CHRONIC RENAL FAILURE RECEIVING CHRONIC HAEMODIALYSIS

	Case No.								
Variable	1	2	3	4	5	6	7	8	Mean ±SD
Hb ($g.dL^{-1}$)	11.0	4.6	8.6	7.5	8.3	8.2	6.6	9.1	8.0 ±1.9
Hct (%)	30	17	26	21	26	24	18	26	24 ±5
Total protein ($g.dL^{-1}$)	68	65	-	53	66	65	63	65	56 ±23
Albumin ($g.dL^{-1}$)	46	39	44	37	39	43	40	41	41 ±3
Urea ($mmol.L^{-1}$)	24	28	28	29	23	24	25	23	26 ±2
Creat. ($\mu mol.L^{-1}$)	1320	1206	1267	1637	1159	1294	1285	1408	1322 ±147
Calcium ($mmol.L^{-1}$)	2.3	2.1	2.3	2.2	2.3	2.5	2.2	2.4	2.3 ±0.1
Inorg. phosphate ($mmol.L^{-1}$)	1.6	1.1	2.2	2.7	1.3	1.8	2.1	1.8	1.8 ±0.5
PTH ($pg.ml^{-1}$)	551	676	341	8	101	172	164	22	254 ±246
Bicarb. ($mmol.l^{-1}$)	19	21	-	14	18	18	15	16	17 ±2
Energy Intake * ($Kcal.day^{-1}$)	1163	1480	-	1627	1676	1400	807	2086	1463 ±405
Energy Intake ($Kcal.kg^{-1}.day^{-1}$)	26	30	-	25	32	27	17	38	28 ±7
Protein Intake ($g.kg^{-1}.day^{-1}$)	0.9	1.0	-	1.0	0.8	1.1	0.7	1.2	1.0 ±0.2
% CHO intake	47	53	-	51	40	38	58	50	48 ±7
% Fat intake	39	33	-	32	49	45	25	35	37 ±8
% Protein intake	14	14	-	17	11	17	17	15	15 ±2

Key: Hb=haemoglobin; Hct=haematocrit; Total protein=serum total protein; Albumin=serum albumin; Creat.=creatinine; Inorg.=inorganic; PTH=serum parathormone; *=total daily energy intake; CHO=carbohydrate.

Note: All blood measures are from samples drawn immediately prior to dialysis.

Light microscopy revealed that all of the patients had atrophied type II fibres (6-62%) while 2 of the patients also had hypertrophy of the type I fibres (51% in patient 6; Table 5.3). The significant changes present were fibre splitting, internalized nuclei, nuclear knots, moth-eaten fibres, fibre degeneration, increased content of lipid droplets and fibre-type grouping (Table 5.3). Myosin ATPase stains on frozen tissue revealed a mean Type I fibre size of 61.2 μm (± 11.8 μm) and a mean Type II fibre size of 46.7 μm (± 11.4 μm) (Table 5.4). Two patients had significant Type II fibre atrophy (mean diameter less than 40 μm - Dubowitz et al., 1985). The mean Type II muscle fibre % was 67 (± 12) (Table 5.4). Two patients displayed Type II fibre predominance (% greater than 80% [Dubowitz et al., 1985]. Table 5.4 lists the morphometric skeletal muscle evaluation of the control subjects, all of which are in the normal range.

Electron microscopy of the muscle samples from 10 of the control subjects had normal ultrastructural features (Figure 5.1); the myocytes contained smoothly outlined nuclei with evenly dispersed chromatin and nuclei of normal size. The mitochondria were regular in shape and size and small subsarcolemmal aggregates were occasionally seen. The myofibrils were aligned and the sarcomeric units showed regular A, Z, H, M and I banding. Glycogen granules were evenly dispersed throughout the cells and no large accumulations were present. The lipid droplets were of normal size and distribution and were usually situated near

the mitochondria. The sarcoplasmic membranes and overlying basement membranes were continuous and evenly contoured. The muscle sample from one control subject revealed foci of myofibrillar loss and the occasional swollen mitochondrion, while another had numerous lipid droplets, subsarcolemmal mitochondrial aggregates and increased glycogen content (Table 5.3).

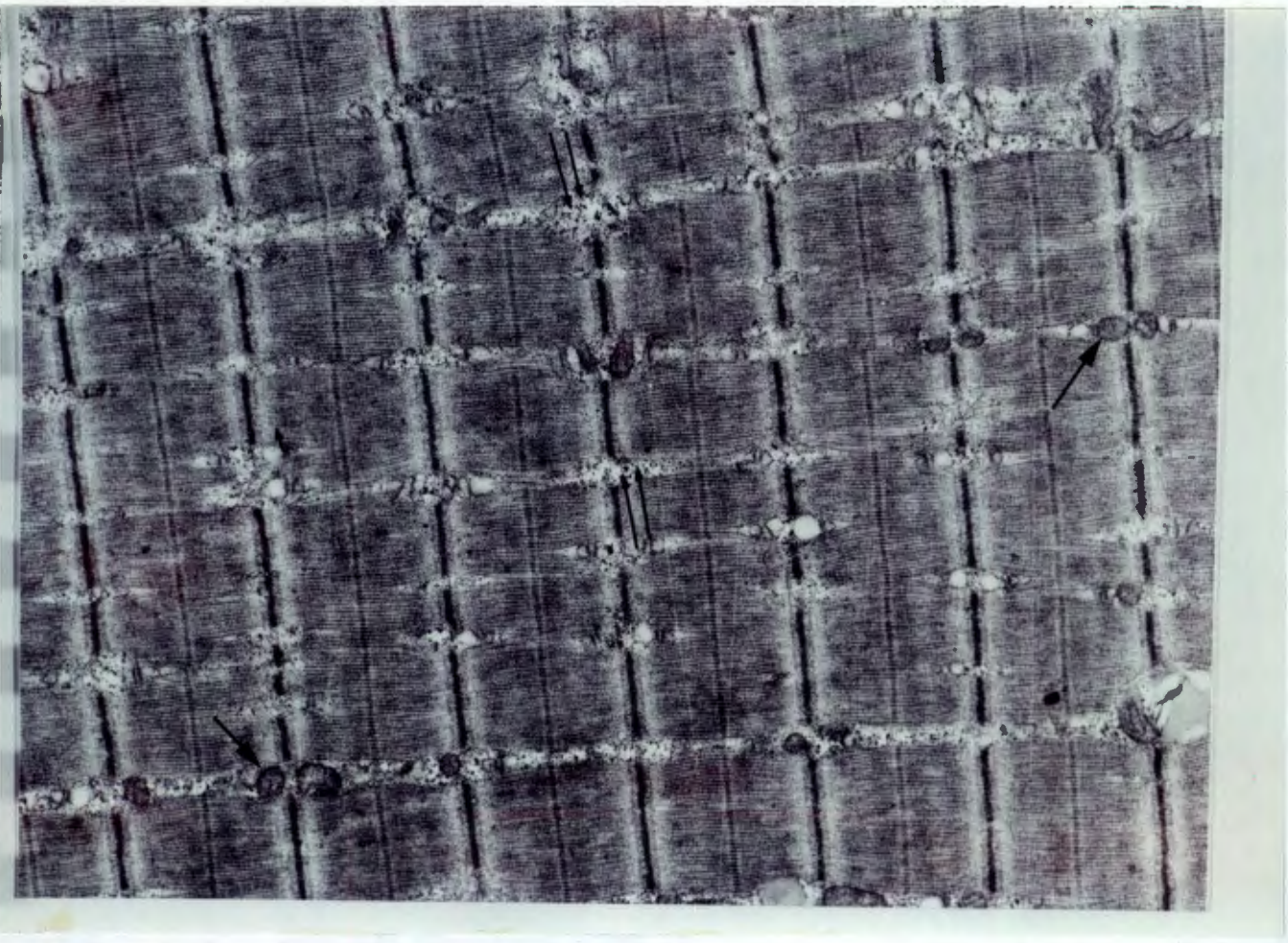

Figure 5.1: Control muscle showing regular myofibrillar arrangement, aligned sarcomeric units, normal mitochondira and evenly dispersed glycogen granules (x 9 750).

TABLE 5.3 MORPHOLOGICAL SKELETAL MUSCLE EVALUATION IN 8 PATIENTS WITH END-STAGE RENAL FAILURE AND 12 CONTROLS.

Case No.	Light Microscopy	Electron Microscopy
1.	Fibre atrophy (60%), fibre splitting, internal nuclei, nuclear chains, moth-eaten fibres, lipid increased, fibre grouping.	Large and pleomorphic mitochondria. Intermyofilamental glycogen aggregation. Fibre haystacking and splitting. Focal myofilamentous loss. Multinucleated.
2.	Type II fibre atrophy (62%), fibre necrosis, severe fibre splitting, numerous internal nuclei, nuclear chains, nuclear knots, moth-eaten fibres, increased lipid, fibre grouping.	Large disrupted mitochondria. Excessive subsarcolemmal glycogen aggregation. Moth-eaten myofilament appearance. Z-band streaming.
3.	Type II fibre atrophy (14%), regenerating fibres, fibre splitting, internal nuclei, moth-eaten fibres.	Elongated and diagonal mitochondria. Excessive subsarcolemmal mitochondrial clusters. Subsarcolemmal glycogen aggregation. Scalloped fibres.
4.	Type II fibre atrophy (32%), fibre splitting, internal nuclei, nuclear chains, fibre grouping.	Pleomorphic and transverse mitochondria. Intermyofilamental glycogen aggregation. Myofibrillar hypertrophy and splitting. Multinucleated and central nuclei.
5.	Type II fibre atrophy (36%), fibre splitting, numerous internal nuclei, nuclear chains and knots, fibre grouping.	Swollen and disrupted mitochondria. Subsarcolemmal clusters of mitochondria. Subsarcolemmal glycogen aggregates. Excessive myofilamentous loss. Multinucleated. Excessive myelin figures.
6.	Type II fibre atrophy (5-10%), hypertrophied Type I and II fibres, myofibre degeneration, fibre splitting, numerous internal nuclei, cytoplasmic inclusion, moth-eaten fibres.	Swollen and disrupted mitochondria. Subsarcolemmal glycogen aggregation. Excessive fibre splitting. Z-band streaming. Multinucleated. Increased dilation of the sarcoplasmic reticulum.

7. Type II fibre atrophy (20%), fibre necrosis, inflammatory cells, internal nuclei, lipid increased.	Swollen, disrupted and transverse mitochondria. Intermyofilamental and subsarcolemmal glycogen aggregates. Excessive myofilamentous loss. Fibrillar bodies.
8. Type II fibre atrophy (6%) and hypertrophied Type I fibres, fibre splitting, internal nuclei, nuclear chains and knots. Lipid increased.	Large, pleomorphic and transverse mitochondria. Intermyofilamental and subsarcolemmal glycogen aggregates. Focal myofilamentous loss. Multinucleated and central nuclei.

Controls

1. Internal nuclei, lipid increase, subsarcolemmal mitochondrial clusters.	Numerous lipid droplets. Increased glycogen in some fibres. Subsarcolemmal mitochondrial aggregates.
2. Type II fibre atrophy, fibre splitting, subsarcolemmal mitochondrial clusters.	Foci of myofibrillar loss. Occasional swollen mitochondria.

Control subjects 3-12 did not show any abnormalities on electronmicroscopy.

TABLE 5.4 MORPHOMETRIC SKELETAL MUSCLE EVALUATION IN 8 PATIENTS WITH END-STAGE FAILURE AND 12 CONTROL SUBJECTS

Case No.	Fibre Size (μm)		% Type II Fibres
	Type I	Type II	
Patients			
1.	60.9 ± 11.2	36.7 ± 9.8**	64
2.	55.4 ± 8.3	44.3 ± 10.0	79*
3.	50.8 ± 10.6	44.4 ± 12.7	68
4.	80.5 ± 14.0	58.9 ± 14.8	68
5.	60.8 ± 10.1	49.3 ± 11.4	69
6.	53.8 ± 10.3	48.9 ± 10.2	52
7.	64.8 ± 8.9	36.5 ± 10.5**	85*
8.	65.2 ± 15.5	56.4 ± 11.8	50
Controls			
1.	74.1 ± 10.6	77.0 ± 12.0	58
2.	62.7 ± 11.1	47.3 ± 14.1	65
3.	73.3 ± 13.0	79.6 ± 14.8	57
4.	81.1 ± 15.5	75.3 ± 15.6	67
5.	60.2 ± 10.0	65.3 ± 9.9	44
6.	62.4 ± 10.1	68.3 ± 8.9	38
7.	68.5 ± 10.4	72.1 ± 13.1	43
8.	70.4 ± 10.9	80.0 ± 14.3	39
9.	57.2 ± 11.3	58.1 ± 8.2	38
10.	65.0 ± 11.5	69.8 ± 10.0	49
11.	57.6 ± 10.1	62.4 ± 9.8	72
12.	59.6 ± 8.0	56.9 ± 9.1	67

Controls 1-5 were sedentary whereas controls 6-12 were athletic males, aged between 20-30 years, who were undergoing athroscopic knee surgery.

Key: * Type II fibre predominance
** Significant Type II fibre atrophy.

In contrast, electron microscopy of myocytes from the renal patients revealed mitochondrial changes, Z-band degeneration and loss of myofilaments in all patients (Table 5.3). These changes which were more widespread and severe than those found in 2 of the control subjects, appeared to be progressive with some cells being more severely affected

than others. Where evidence of severe mitochondrial damage was found, the myofilamentous changes were also more marked.

Mitochondria showed a spectrum of changes. These included the formation of either paracrystalline or angular electron dense (Figures 5.2 and 5.3) intra-mitochondrial inclusions. Bizarre branching mitochondria were also seen; frequently these were arranged at right angles to the sarcomeres (Figure 5.4). Extensive myofilament and Z-band lysis with associated swollen and disintegrated mitochondria containing occasional myelin figures and loss of christae was also a common feature (Figure 5.5). Myocytes showing evidence of mitochondrial damage showed accumulation of glycogen granules (Figure 5.6).

Myofibre changes varied from Z-band streaming to loss of Z-bands and myofilaments (Figure 5.7). The severity of the myofibre loss varied in proportion to the severity of the mitochondrial damage. Thus, where severe loss of myofilaments was found, mitochondria were severely swollen or degenerate.

Evidence for muscle regeneration was present. Regenerated myocytes showed multiple nuclei with characteristic margination of chromatin and prominent nucleoli (Figure 5.8). Occasional multi-nucleated primitive cells revealed their myoblastic nature by the presence of scanty myofilaments and irregular primitive Z-bands (Figure 5.9). The degenerative and regenerative changes were frequently

found side by side, indicating an ongoing process of damage and repair.

Figure 5.2 A mitochondrion containing an elongated "paracrystalline-like" inclusion (arrow) (x 54 675).

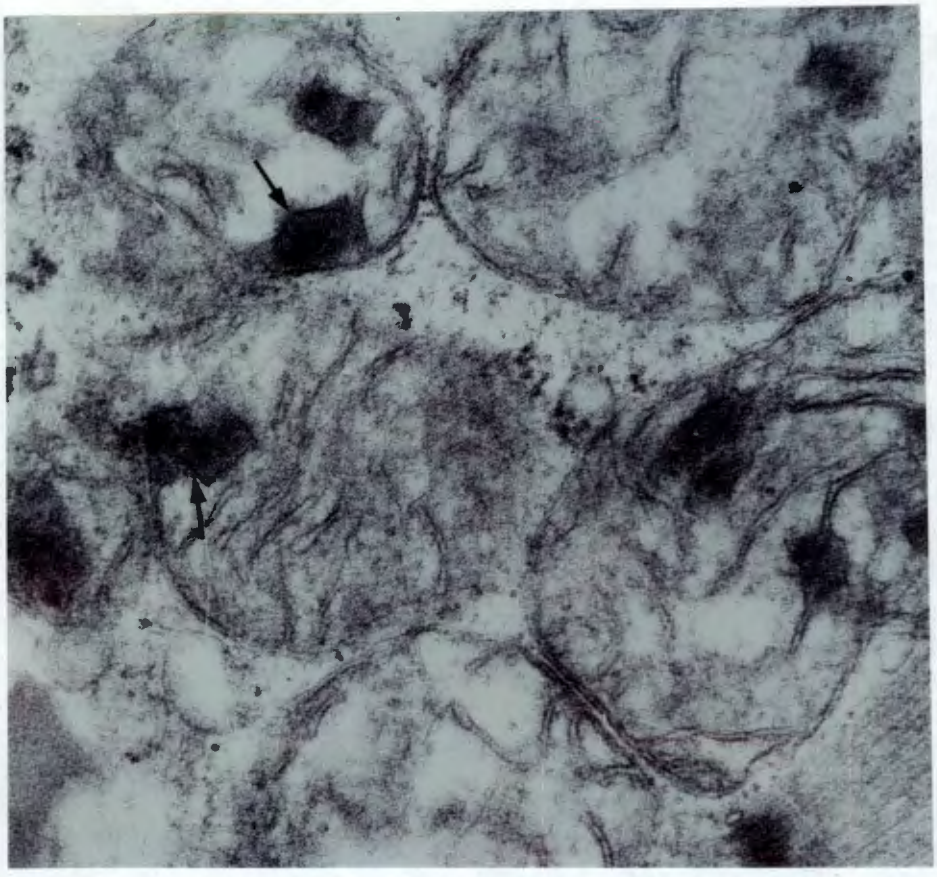

Figure 5.3. Mitochondria showing angular, electron dense inclusions (arrows) (x48 600).

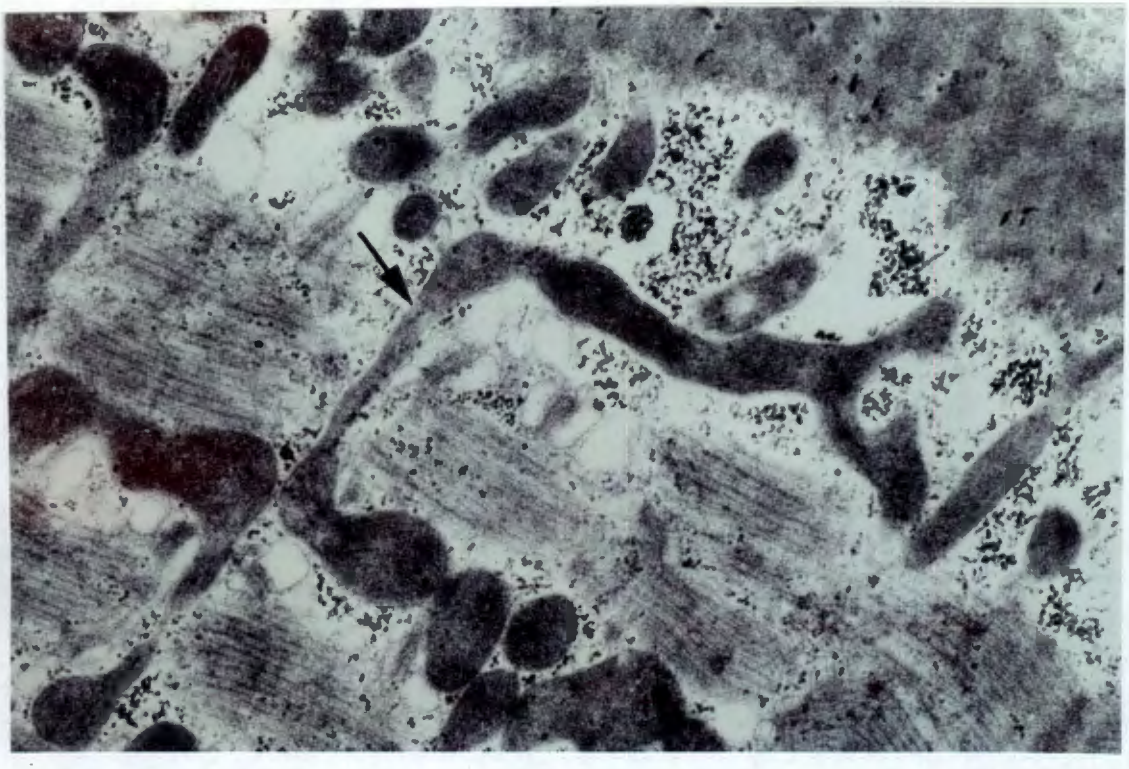

Figure 5.4. Large branching, pleomorphic mitochondria which also transverse the myofilaments (x 19 200).

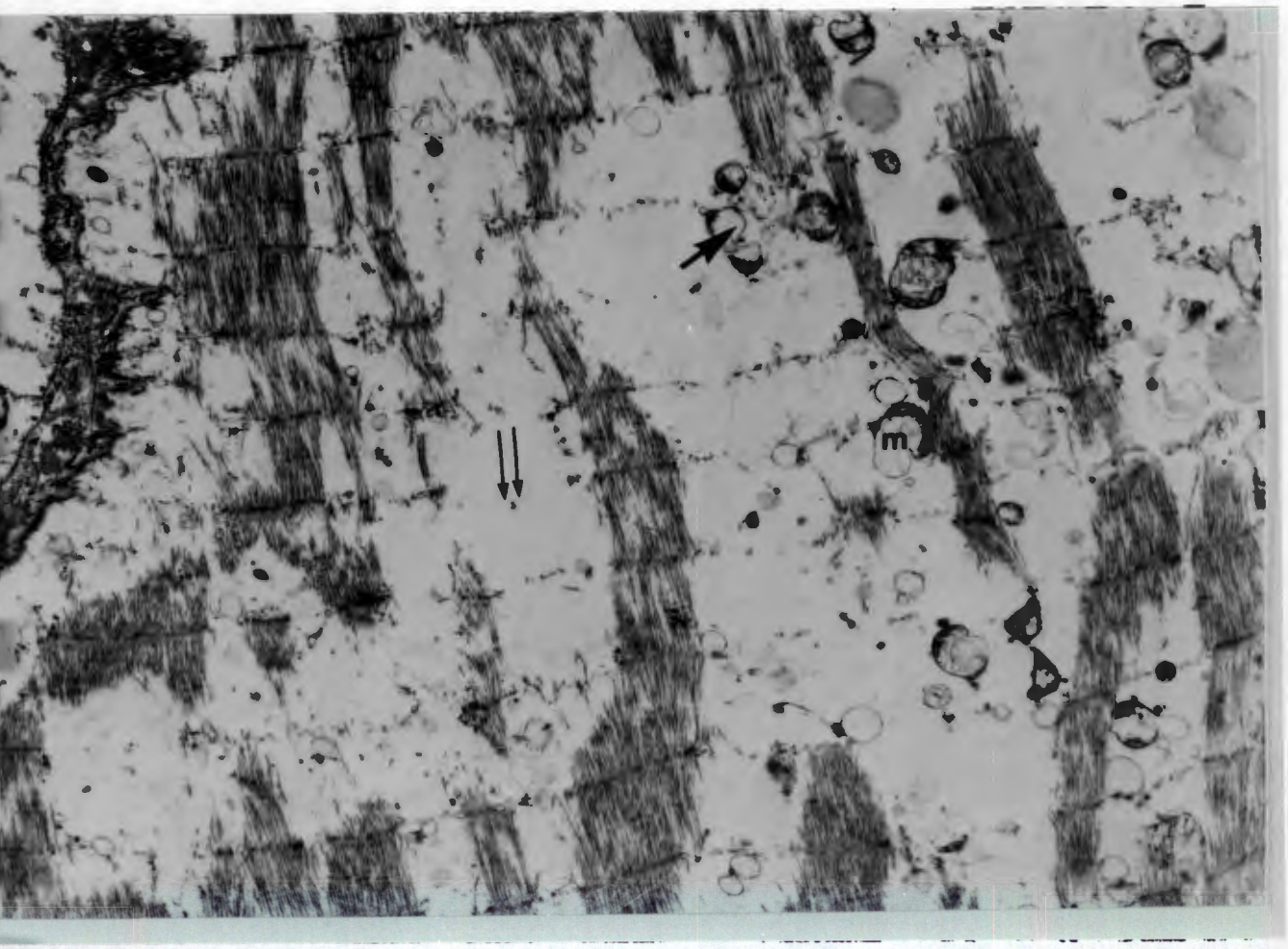

Figure 5.5. Severe myofilament and Z-band dissolution is evident. The mitochondria are swollen, disrupted and contain myelin whorls and a paucity of christae (arrows) (x 10 430).

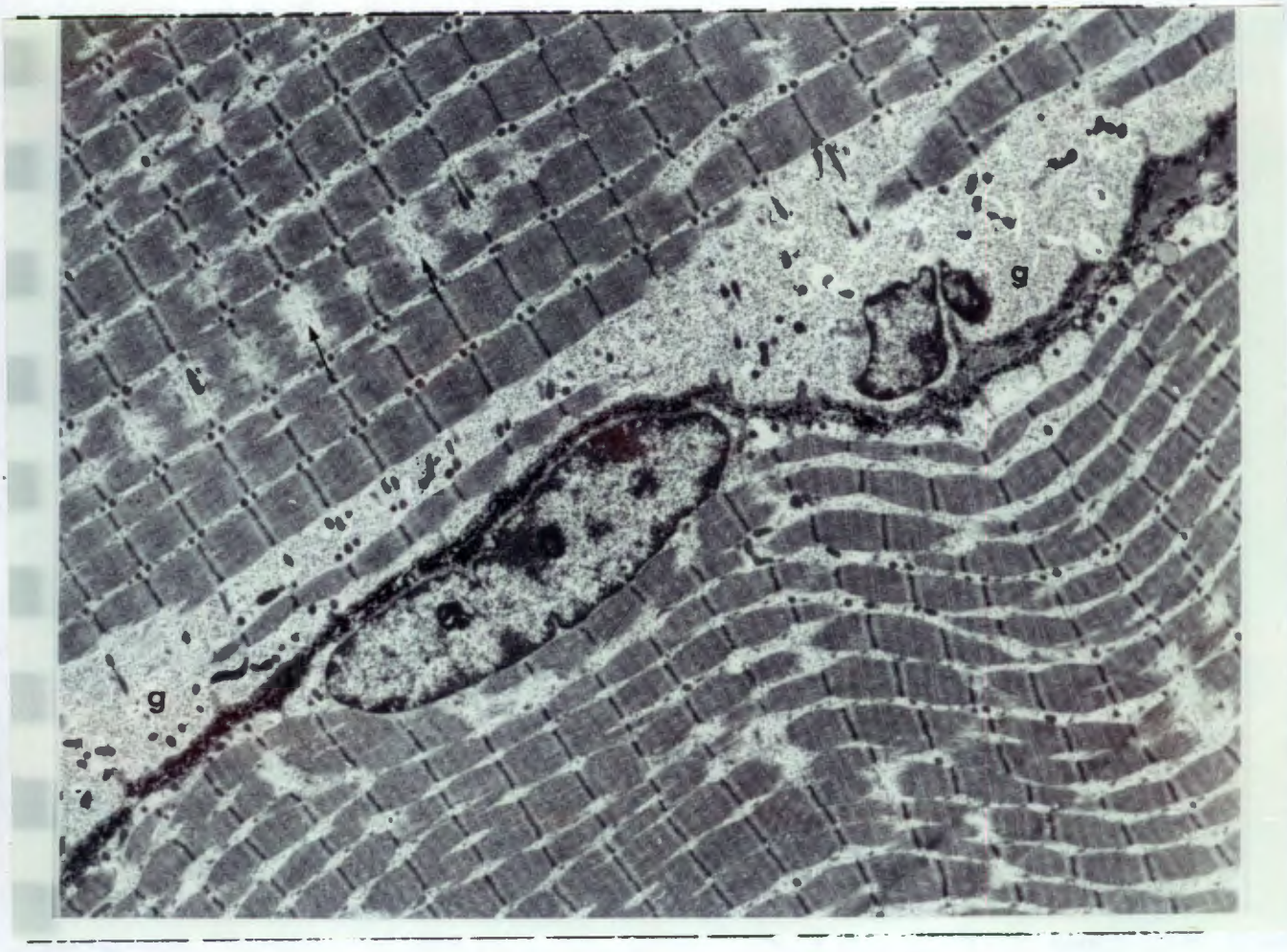

Figure 5.6. Intermyofibrillar and large sub-sarcolemmal glycogen (g) aggregates are present. Z-band lysis (arrows) is also evident (x 6 825).

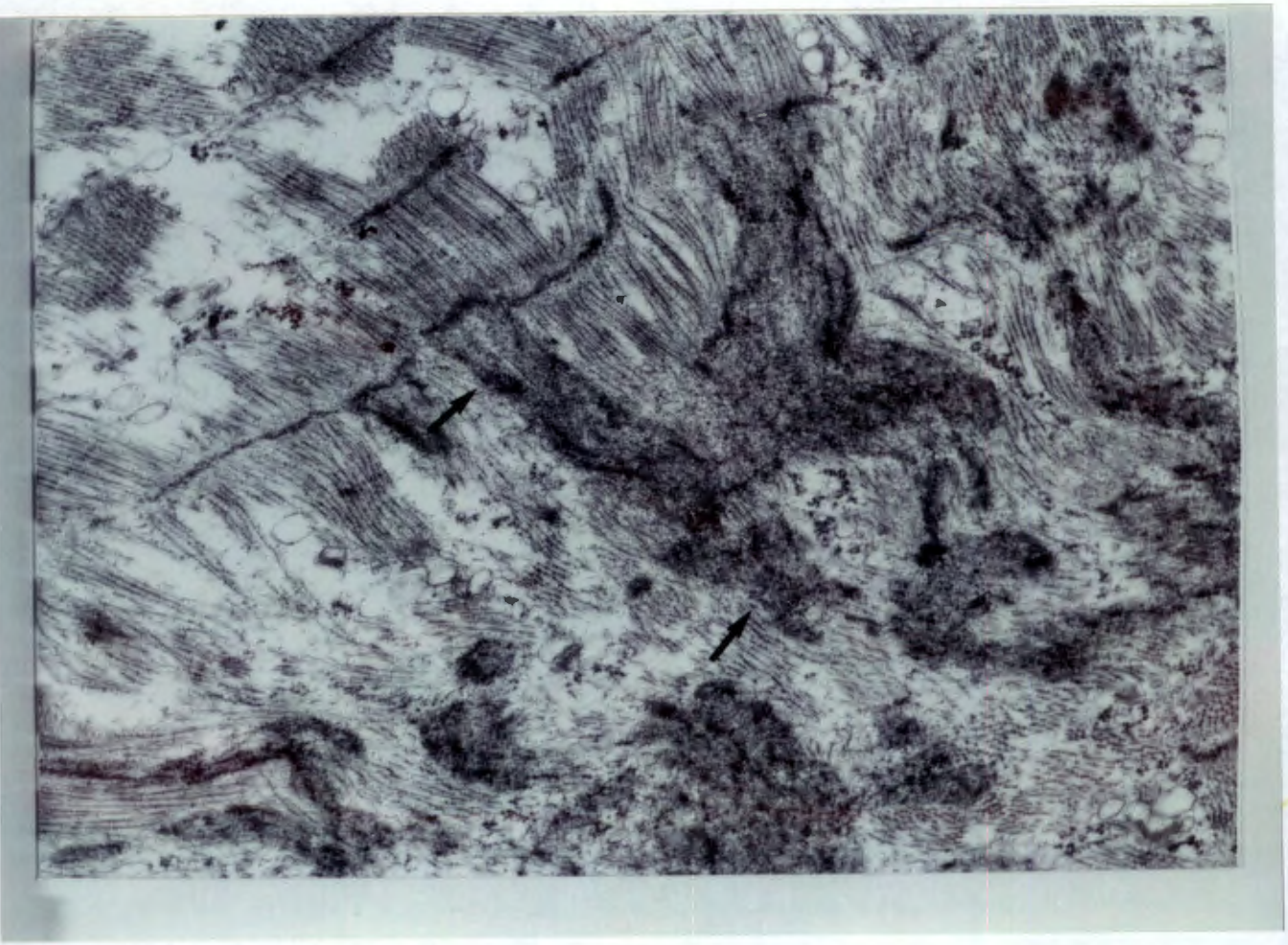

Figure 5.7. Myofibrillar disorganization with Z-band streaming. Z-band and myofilament loss is also present (x 22 400).

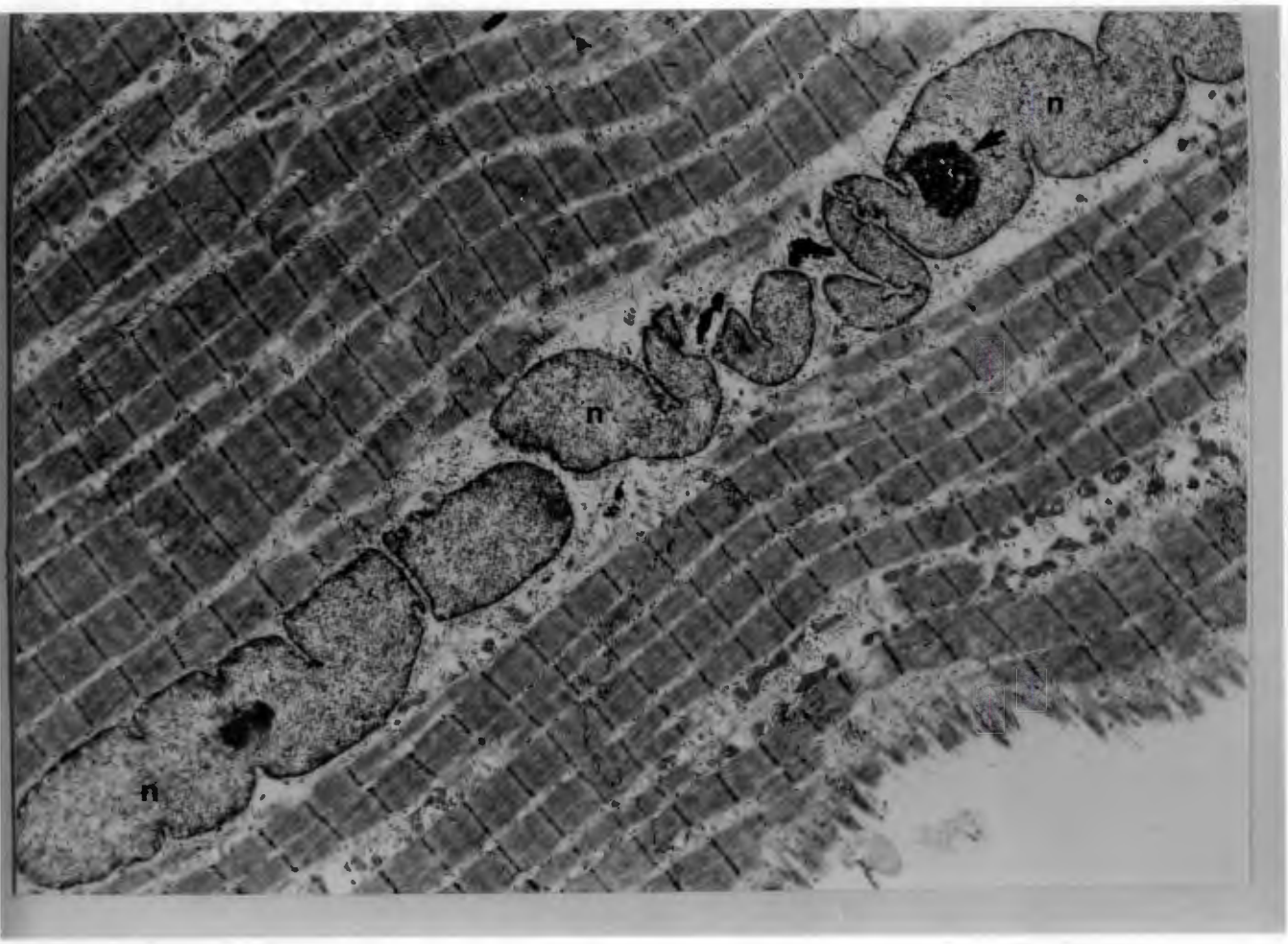

Figure 5.8. A myocyte with several tandem nuclei containing prominent nucleoli (x 6 825).

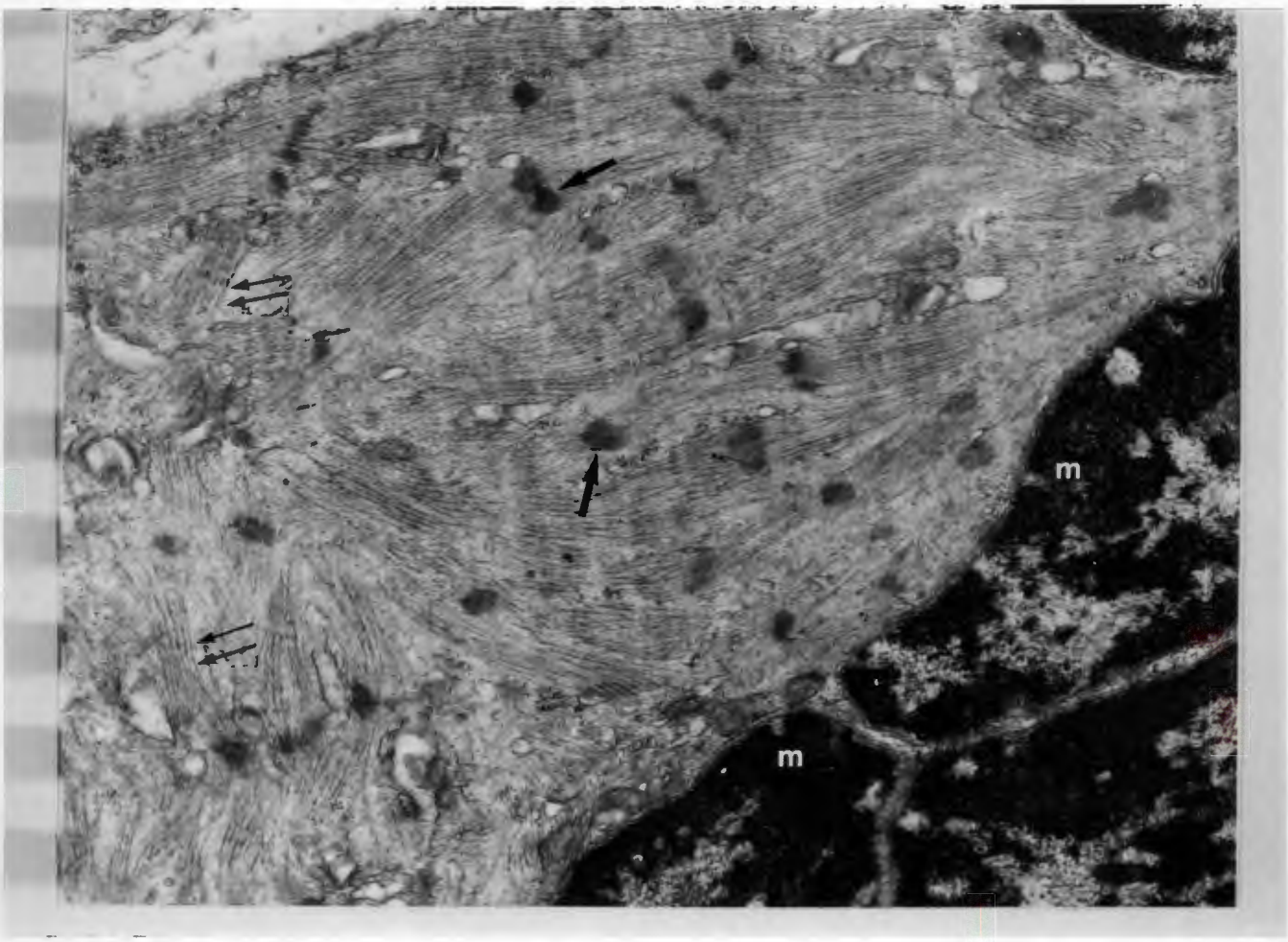

Figure 5.9. A multinucleated myofibroblast displaying large nucleoli, irregular myofilament arrangements and small, immature and randomly orientated Z-band (arrows) (x 8 940).

DISCUSSION

In a previous study from this laboratory [Kempeneers et al., 1990] the skeletal muscles of renal transplant recipients were shown to have a preponderance of Type II fibres with low oxidative capacity. Patients with congestive heart failure also show this preponderance [Lipkin et al., 1988].

The present study found these changes in only 2 of the 8 patients receiving chronic haemodialysis. Further, muscle fibre size and distribution was not different from the normal population [Doriguzzi et al., 1984]. Skeletal muscle oxidative capacity was reduced compared to values measured in athletes but was not lower than values measured in physically trained renal transplant patients [Kempeneers et al., 1990] suggesting that this abnormality may not be reversed by either renal transplantation or exercise training. However this specific technique provides a relatively less sensitive measure of skeletal muscle oxidative capacity. Perhaps more sensitive measures including analysis of specific oxidative enzyme activities, may be required to accurately quantify the extent of any abnormality in skeletal muscle oxidative capacity in these patients and its response to renal transplantation or exercise training, or both.

However the important contribution of this study is to show significant changes on light and electron microscopy in muscle samples taken from these patients. Light microscopic

changes included fibre splitting, internalized nuclei, fibre degeneration and regeneration, increased content of lipid droplets and fibre-type grouping. Electronmicroscopy revealed mitochondrial and myofilamentous changes in these patients (Table 5.3); changes that were not present in 10 of the 12 control subjects sampled under identical conditions. In particular, there was extensive myofilament and Z-band lysis (Figure 5.7), glycogen accumulation (Figure 5.6) and evidence of regeneration (Figures 5.8 and 5.9). Mitochondria with bizarre shapes (Figure 5.4), with inclusions (Figures 5.2 and 5.3) and loss of christae (Figure 5.5) were common. Abnormalities present in 2 of the control subjects were of a minor nature (Table 5.3).

Many of the mitochondrial changes seen are non-specific and their morphology does not offer a clue to their pathogenicity. Similar changes have been described in patients with intermittent claudication (Teravainen et al., 1977) and in tourniquet-induced ischaemia (Mortimer et al., Unpublished observations). However, mitochondria with branching forms and unusual orientation across the sarcomeres have not to our knowledge, been reported previously.

Some of the morphological abnormalities reported in this paper were also found by Floyd et al. (1974). They found Type II fibre atrophy and non-specific degenerative changes including numerous foci of Z-band degeneration; small foci

of myofibre necrosis; complex lipid vacuoles, and sub-sarcolemmal mitochondrial aggregates.

Similarly the increase in muscle glycogen content reported in this study has also been found in some [Floyd et al., 1974; Nakao et al., 1982] but not all previous studies [Bergstrom et al., 1969].

The explanation for these changes are not clear. Floyd et al. (1974), suggested that many factors including acidosis, abnormalities in vitamin D metabolism or in serum calcium concentrations, or renal haemodialysis itself may explain these abnormalities. Elevated serum parathyroid hormone (PTH) concentrations have been shown to enhance muscle proteolysis, to impair muscle oxidative capacity [Smorgorzewski et al., 1988] and to be associated with intra-mitochondrial inclusions [Ghadially FN, 1975] similar to those seen in this study. However, not all of the patients in this study had elevated serum PTH concentrations (Table 5.1), although all had mitochondrial abnormalities.

Other possible explanations include prolonged inactivity and dietary deficiencies in particular, an inadequate protein or calorie intake, or inappropriate haemodialysis schedules.

However the majority of inactive controls in this study did not show any of these abnormalities (Table 5.3) nor have such abnormalities been reported in healthy but inactive subjects [Dubowitz et al., 1985]. In addition, protein

intake was adequate and serum total protein and albumin concentrations were normal in these patients suggesting that severe dietary deficiencies were not present in the total group of patients. Furthermore, blood biochemical measures taken immediately prior to dialysis (Table 5.2) and on which decisions regarding the dialysis schedule are based, were within the norms for this Renal Unit.

How might these morphological abnormalities explain the impaired exercise tolerance of these patients? Exercise in patients with congestive heart failure is associated with an abnormally large reduction in phosphocreatine (PCr) concentrations with an increased intracellular acidosis [Massie et al., 1987]. The same abnormalities have recently been reported in patients with end-stage renal failure [Nishida et al., 1991] who also showed delayed recovery of muscle pH after exercise. These changes are compatible with an impaired capacity for oxidative metabolism which presumably results from the mitochondrial abnormalities reported in this study.

Despite this finding that muscle pH falls more rapidly during low intensity exercise in patients with end-stage renal failure, this cannot be the sole reason why their exercise tolerance is impaired. For patients with end-stage renal failure who have either undergone renal transplantation [Kempeneers et al., 1990] or who are receiving chronic haemodialysis [Chapter 3], or who have also received EPO therapy [Chapter 4] all terminate maximum

exercise at low blood lactate concentrations. Nor do they develop even a mild acidosis during maximal exercise [Lundin et al., 1987]. This indicates that the impaired skeletal muscle oxidative capacity measured in both groups, and the mitochondrial abnormalities identified in these renal haemodialysis patients, does not limit their exercise tolerance by increasing their reliance on "anaerobic" glycolysis or with the development of a marked acidosis [Lundin et al.,1987]. Hence the exact mechanism whereby the mitochondrial and myofilamentous abnormalities identified in these patients, might impair their exercise tolerance remains to be established.

In summary, this study establishes that the skeletal muscles of patients receiving chronic haemodialysis show specific abnormalities, in particular multiple mitochondrial abnormalities, excessive glycogen content and marked myofilamentous loss. Further, the damage appears to be ongoing with degenerative changes being found in fibres adjacent to others showing evidence of regeneration.

Although this study cannot offer an explanation for the pathogenicity of the myopathy of renal failure, we have demonstrated morphological and biochemical changes in the muscle which may be sufficiently severe to account for the muscle weakness and severely impaired exercise tolerance found in all these patients [Painter et al., 1986(b); Barnea et al., 1980; Gutman et al., 1981; Lundin et al., 1987; Kempeneers et al., (1990); Chapter 3; Chapter 4].

SUMMARY

Chapter 3 suggested that impaired function of skeletal muscle limits the exercise tolerance of patients with end-stage renal failure who are maintained on haemodialysis.

To study the morphology of the condition, muscle biopsies were performed on eight patients with renal failure-associated myopathy. Control samples were taken from seven healthy athletes undergoing knee surgery and from five otherwise healthy but untrained subjects.

Tissues were examined by routine light and transmission electron microscopy. Histochemical staining of frozen sections for ATPase and quantitative computer-assisted morphometry of the fibre type and size was performed. Mean (±SD) size for Type I fibres in patients was 61.2 (±11.8 μm) while Type II fibres measured 46.7 (±11.4 μm). The mean Type II fibre % was 67 (±12%). These values are within the normal population range and were not different from controls. Significant changes were found on light microscopy of patient samples. These included fibre splitting, internalized nuclei, nuclear knots, moth-eaten fibres, fibre degeneration and regeneration, increased content of lipid droplets and fibre-type grouping. Electron microscopy showed a large variety of non-specific abnormalities including mitochondrial changes, Z-band degeneration, myofilament loss and accumulation of intra-

cellular glycogen. Ten of 12 control subjects showed no such changes; minor abnormalities were noted on both light and electron microscopy in the remaining 2. Muscle oxidative capacity (QO_2 = 19.5 ± 5.1 fL $O_2.min^{-1}$) for patients with end-stage renal failure was not different from values for those who had undergone renal transplantation, but was lower than values in trained athletes.

Thus patients undergoing chronic haemodialysis for chronic renal failure have significant morphological abnormalities in skeletal muscle. Such changes might explain the impaired skeletal muscle function of these patients.

CHAPTER 6

THE EFFECTS OF ATENOLOL OR RECOMBINANT HUMAN ERYTHROPOIETIN ON THE EXERCISE TOLERANCE OF PATIENTS WITH END-STAGE RENAL FAILURE RECEIVING CHRONIC HAEMODIALYSIS

INTRODUCTION

Adequate haemodialysis is often insufficient to correct the hypertension and anaemia associated with end-stage renal failure [Chobanian et al., 1982; Maher, 1989(a)]. Therefore, additional medication may be necessary such as ß-blockers and EPO, respectively. But medications such as ß-blockers reduce the peak exercise heart rates and VO_2 max of healthy subjects [Van Baak, 1988]. In contrast, EPO increases haemoglobin concentrations and VO_2 peak in patients with end-stage renal failure [Mayer et al., 1988; Chapter 5].

Low peak exercise heart rates and haemoglobin concentrations are the two factors most commonly cited in the argument for a central (cardiovascular) limitation to exercise in patients with end-stage renal failure receiving chronic haemodialysis [Chapter 1]. Patients who require either medication may therefore demonstrate peak exercise heart rates or haemoglobin concentrations significantly different from patients not using these drugs. Thus conclusions reached regarding factors which limit patients with end-stage renal failure receiving chronic haemodialysis during peak exercise may be erroneous, if patients receiving exercise affecting medications are grouped together with results from patients who are not using such medications.

This study was designed, therefore, to investigate the possible effects of non-selective grouping of these patients in exercise-related studies. In particular the grouping of those patients who are ingesting either Atenolol (100mg.day^{-1}) or receiving EPO therapy, with patients who are on neither form of medication.

MATERIALS AND METHODS

Patient selection. Thirty-seven medically stable adult (30 male and 7 female) patients with chronic end-stage renal failure, receiving chronic haemodialysis volunteered for the study which had been approved by the Committee for Research on Human Subjects of the University of the Witwatersrand. Patients were from the Renal Units of the J.G. Strijdom, Johannesburg General and Baragwanath Hospitals, and were all screened by their respective renal physicians prior to entering the study. Informed consent was obtained from each patient after detailed description of the protocol. The sample of patients was subdivided into three groups according to their medication. Group A (n=9) were those patients on EPO, Group B (n=7) those on ß-blockers (all were receiving 100mg.day^{-1} dosages of Atenolol) and Group C (n=21) those on neither EPO nor ß-blockers [Table 6.1]. Most patients were receiving phosphate binders (Actal. R) and calcium supplements (Titralac. R); neither of which are considered to influence exercise performance. Nine patients were on either aspirin or persantin. Ten patients were receiving the calcium blocker (Nifedipine); two of these patients were in Group A, five in Group B and three in Group C.

Exercise tests. Each patient performed a graded exercise test to exhaustion for measurement of peak oxygen consumption ($VO_{2\ peak}$) as well as an isokinetic exercise test for measurement of peak torque at three different

contraction velocities. The patients were required to perform exactly the same test protocols as those described in Chapter 3.

Values for haemoglobin concentration and haematocrit were obtained, prior to any testing, from each patient's files at the respective renal haemodialysis units.

Statistical analysis

This study is a cross-sectional comparison of 3 groups. Data are expressed as mean ± SD. Any differences between the groups were assessed using 1-way ANOVA. If a significant difference ($p<0.05$) between the Groups was found, then a Bonferroni procedure was used to determine were the difference lay.

RESULTS

Group A (patients on EPO) consisted of 9 (6 male and 3 female) patients, 25% of the total sample size. Group B (patients on ß-blockers) consisted of 7 (5 male and 2 female) patients, 19% of the total sample, whilst Group C consisted of 21 (19 male and 2 female) patients, 56% of the total sample.

Anthropomorphic and haematological measurements are reported in Table 6.1. There were no significant differences for age, height, weight, duration of haemodialysis, haemoglobin concentration, or haematocrit between groups.

Table 6.1. Anthropomorphic and Haematological Measurements in the Three Groups of Patients with End-Stage Renal Failure

	Groups		
	A	B	C
n	9	7	21
Age (yrs)	38 ± 9	38 ± 11	39 ± 10
Height (cm)	169 ± 9	167 ± 11	169 ± 9
Weight (kg)	65 ± 13	66 ± 11	61 ± 11
Dialysis Duration (mo)	54 ± 52	23 ± 44	23 ± 32
Hb ($g.dL^{-1}$)	8.1 ± 1.7	7.5 ± 0.7	8.3 ± 2.6
Hct (%)	25 ± 6	23 ± 2	25 ± 8

Data are expressed as mean ± SD.
ABBREVIATIONS: mo=months; Hb=haemoglobin; Hct=haematocrit
Group A = patients on EPO; Group B = patients on ß-blockers; Group C = patients requiring neither medication. None of the variables in Table 1 was significantly different ($p>0.05$)

The causes of renal failure are reported in Table 6.2. Hypertension was the leading cause of renal failure in all the groups.

Table 6.2. Causes of Renal Failure in Three Groups of Patients Receiving Chronic Haemodialysis

	Groups		
	A	B	C
Secondary to Hypertension	2	2	7
Chronic Glomerulonephritis	2	1	5
Unknown	-	2	4
Reflux Nephropathy	-	-	3
Analgesic Nephropathy	1	1	-
Idiopathic Nephritic Syndrome	1	-	-
Chronic Pyelonephritis	1	-	-
Chronic Lupus Nephritis	1	-	-
Traumatic Bilat. Nephrectomy	1	-	-
IgA Nephropathy	-	1	-
Recurrent FSH	-	-	1
Polyarthritis	-	-	1
Total	9	7	21

ABBREVIATIONS: Bilat.=Bilateral; FSH=Focal Segmental Hyalinosis. Group A = patients on EPO; Group B = patients on ß-blockers; Group C = patients requiring neither medication

Table 6.3 lists cardiorespiratory variables measured at rest and during maximum exercise to exhaustion. Peak oxygen consumption was significantly lower ($p<0.05$) in Group B compared to Groups A and C (19.2 ± 2; 15 ± 3.5; and 20.1 ± 5 ml $O_2.min^{-1}.kg^{-1}$ for Groups A; B; and C respectively). Resting heart rates were also significantly lower ($p<0.05$) in Group B compared to Groups A and C (88 ± 11; 72 ± 8; 86 ±14 beats.min^{-1} for Groups A; B; and C respectively) as were peak heart rates (158 ± 18; 110 ± 19; 152 ± 16 beats.min^{-1} for Groups A; B; and C respectively).

However, no significant differences were found between peak workrate, peak ventilation, blood lactate concentrations at rest or at peak exercise, and respiratory exchange ratios.

The predominant subjective reason for terminating the VO_2 peak exercise test was leg fatigue; other reasons included chest discomfort, not considered to be angina.

Table 6.4 lists peak isokinetic muscle strength, expressed as peak torque, in ft.lbs, was measured using the Cybex II Isokinetic Dynamometer. No significant differences were found at any of the limb contraction velocities between any measurements in any of the groups.

Table 6.3. Cardiorespiratory and Metabolic Parameters at Rest and during Peak Exercise in Three Groups of Patients Receiving Chronic Haemodialysis

	Groups A	B	C	Sig.
n	9	7	21	
VO_2 peak ($mL\ O_2.kg^{-1}.min^{-1}$)	19.2 ± 2.0	15.0 ± 3.5	20.1 ± 5	<0.05*
Workrate (Watts)	98 ± 19	80 ± 19	101 ± 28	NS
VE PEAK ($L.min^{-1}$)	72.7 ± 30	56.0 ± 12	69.7 ± 22	NS
Heart Rate				
REST	88 ± 11	72 ± 8	86 ± 14	<0.05*
PEAK	158 ± 18	110 ± 19	152 ± 16	<0.05*
($beats.min^{-1}$)				
RQ				
REST	0.84 ± 0.08	0.78 ± 0.05	0.80 ± 0.08	NS
PEAK	1.10 ± 0.07	1.10 ± 0.06	1.14 ± 0.08	NS
Lactate				
n	9	6	15	
REST	1.86 ± 0.47	1.71 ± 0.63	1.88 ± 0.51	NS
PEAK	5.31 ± 2.65	3.09 ± 0.46	4.94 ± 2.22	NS
($mmol.L^{-1}$)				

Data expressed as mean ± SD.
ABBREVIATIONS: VO_2 peak=Peak oxygen consumption; VE PEAK=Peak minute ventilation; RQ=Respiratory quotient; NS=not significant ($p>0.05$); *=significant differences were between Group A vs B and Group C vs B but not between Group A vs C. Group A=patients on EPO; Group B=patients on ß-blockers; Group C=patients requiring neither medication

Table 6.4. Peak Isokinetic Quadriceps Muscle Strength in Three Groups of Patients Receiving Chronic Haemodialysis

	Groups A	B	C
n	9	7	21
60 (ft.lbs)	89 ± 35	95 ± 21	98 ± 26
180 (ft.lbs)	59 ± 27	63 ± 18	65 ± 20
240 (ft.lbs)	51 ± 25	55 ± 14	56 ± 18

Data expressed as mean ± SD. Limb contraction velocities measured in degrees.second^{-1}. Peak torque measured in foot.pounds (ft.lbs). No significant differences were found between any of the groups ($p>0.05$).
ABBREVIATIONS: Group A=patients on EPO; Group B=patients on ß-blockers; Group C=patients requiring neither medication

Correlation coefficients for $VO_{2\ peak}$ and variables relating to either isokinetic muscle function or blood oxygen carrying capacity can be found in Table 6.5. The only positive significant correlations ($p<0.05$) were found in Group C relating $VO_{2\ peak}$ to isokinetic muscle strength. No significant correlations ($p>0.05$) were found between $VO_{2\ peak}$ and haemoglobin concentration.

TABLE 6.5 CORRELATION COEFFICIENTS FOR PEAK OXYGEN CONSUMPTION AND VARIABLES RELATING TO EITHER ISOKINETIC MUSCLE FUNCTION OR BLOOD OXYGEN CARRYING CAPACITY

Group		A		B		C	
	n	(9)		(8)		(21)	
		Values		Values		Values	
		r	p	r	p	r	p
Variable							
Isokinetic Muscle Strength							
at 60 deg.sec^{-1}		.43	NS	.32	NS	.47	<0.05
at 180 deg.sec^{-1}		.41	NS	.22	NS	.49	<0.05
at 240 deg.sec^{-1}		.42	NS	.36	NS	.49	<0.05
Blood Oxygen Carrying Capacity							
Hb concentration		.02	NS	.53	NS	.31	NS

ABBREVIATIONS: deg.sec^{-1}=degrees per second; NS=not significant ($p>0.05$); Hb=haemoglobin concentration.
Group A=patients on EPO; Group B=patients on ß-blockers;
Group C=patients requiring neither medication

DISCUSSION

This study included any patient who was willing and medically able to undergo the testing procedures. The finding that 25% and 19% of the total sample size had either been treated with EPO or were ingesting ß-blocker medication respectively and that the leading identifiable cause of renal failure was failure secondary to hypertension indicates that the effects of these medications on the exercise tolerance of patients receiving chronic haemodialysis should not be ignored.

All 3 groups were matched according to age, height, weight, duration of haemodialysis, haemoglobin concentration and haematocrit. However, patients in Group B were on 100mg atenolol.day^{-1} and patients in Group A were on maintenance doses of EPO whereas patients from Group C were receiving neither medication.

The novel finding of this study was that patients receiving ß-blockers terminated exercise at significantly lower $VO_{2\ peak}$ values and heart rates than did patients not receiving ß-blockers. These findings imply that results from exercise-related studies may be altered if these studies fail to take account of the grouping of patients who are on ß-blockers with those who are not. This is especially the case if conclusions are to be made with regard to the $VO_{2\ peak}$ and to factors limiting exercise tolerance in patients with end-stage renal failure.

In particular, several studies have reported significant improvements in exercise tolerance after a period of training. But these studies also report a reduction in the number of patients using ß-blockers [Goldberg et al., 1980; Hagberg et al., 1983; Goldberg et al., 1983]. The findings of this study suggests that a significant percentage of the improvements in exercise tolerance may be due to a change in medication and may not be entirely due to a training effect.

Ten percent of patients with end-stage renal failure with hypertension cannot be treated by adequate haemodialysis alone [Chobanian et al., 1982]. Atenolol, 100mg daily, is often the treatment of choice in those patients whose hypertension does not respond satisfactorily to haemodialysis [Maher, 1989(b)]. ß-Blockers produce a reduction of heart rate, thereby potentially reducing cardiac output, at rest and during exercise [Van Baak, 1988]. This reduction of cardiac output can lead to impaired muscle blood flow [Saltin, 1990] which in turn reduces the amount of oxygen reaching the working muscles. Thus maximal exercise may be adversely affected by medications, such as ß-blockers, which reduce cardiac output and skeletal muscle blood flow.

Peak isokinetic muscle strength, measured during a very brief period of exercise, is probably independent of muscle blood flow and would thus be unaffected by the reduction of muscle blood flow described above. Hence, it is not

surprising that no significant differences in peak muscle strength were found between the groups.

In Chapter 3, we concluded that isokinetic muscle strength predicts VO_2 peak in patients with end-stage renal failure who are receiving chronic haemodialysis. However, since there were no significant differences in isokinetic muscle strength between the three groups yet VO_2 peak was lower in Group B, ß-blockers must produce some effect during more prolonged exercise which decreases VO_2 peak. One possibility for this is that the VO_2 peak test measures two variables, one of which relates to muscle strength and is predicted by the isokinetic muscle strength tests. However as the VO_2 peak lasts for some time, an element of fatigue resistance [Coetzer et al., 1993) may be involved. Perhaps ß-blocking agents increase the rate at which fatigue develops during exercise so that persons using these agents terminate exercise prematurely, thereby reaching lower VO_2 peak values. Furthermore, significant correlations between VO_2 peak and isokinetic muscle strength were found only in Group C. This finding, as well as the absence of a significant correlation between VO_2 peak and haemoglobin concentration in any of the 3 groups, confirms our previous conclusions in Chapter 3.

CONCLUSIONS

A large proportion of patients undergoing chronic haemodialysis require EPO therapy or ß-blockers to control their anaemia and hypertension respectively. Since it has been suggested that the exercise tolerance of these patients may be limited by a reduced cardiac output, from low heart rates, and low arterial content, from anaemia, we decided to study the effects of medications which influence both of these factors on their VO_2 peak.

The results of this study clearly show that patients with end-stage renal failure undergoing maintenance haemodialysis and receiving ß-blockers have significantly lower peak heart rates, measured during exhaustive exercise, than patients not receiving ß-blockers.

Thus conclusions concerning the role that low peak exercise heart rate has in limiting the exercise tolerance of these patients must be viewed with caution, if the sample includes patients receiving ß-blocker medication. In a recent study, Moore et al. 1993, despite acknowledging that propanolol biased the heart rate results of 2 of their patients (20% of the total sample size) included their results in the final analysis. These authors later concluded that exercise tolerance was primarily limited by low peak heart rate and anaemia.

Once their haemoglobin concentrations are corrected, patients requiring partial correction of their anaemia attain exercise tolerance levels similar to patients not requiring correction. Hence inclusion of patients receiving EPO in exercise-related studies appear not to adversely alter the results.

However, it appears as if ß-blockers can compromise already extremely compromised $\dot{V}O_{2\ peak}$ levels of patients undergoing chronic haemodialysis.

It is also suggested that any future studies which aim to unravel the multitude of possible causes for the low exercise tolerance of patients undergoing chronic haemodialysis should not include patients on ß-blockers in their analysis.

SUMMARY

Hypertension and anaemia occur commonly in patients with end-stage renal failure undergoing chronic haemodialysis. Both hypertension and anaemia are often not controlled by adequate haemodialysis or blood transfusions, respectively. Atenolol, 100mg daily, is a ß-blocker which is often the treatment of choice for the hypertension in patients receiving chronic haemodialysis. Recombinant human erythropoietin (EPO) has proved successful in both partially correcting the anaemia associated with end-stage renal failure and dramatically improving the exercise tolerance of these patients, as shown in Chapter 5.

Both Atenolol and EPO are capable of influencing VO_2 peak during maximal exercise. This study was designed, therefore, to investigate the possible effects of non-selective grouping of these patients in exercise-related studies. In particular patients were grouped into those receiving EPO (Group A); or ingesting Atenolol ($100mg.day^{-1}$) (Group B) or patients on neither form of medication (Group C).

Patients were assigned to one of three groups according to their medications. Group A (n=9) were patients who were on EPO; Group B (n=7) were patients on Atenolol 100mg daily; and Group C (n=21) were patients on neither Atenolol nor EPO.

Peak oxygen consumption [Group A 19.2 ± 2.0; Group B 15 ± 3.5; and Group C 20.1 ± 5 mL $O_2.kg^{-1}.min^{-1}$] and peak heart rates [Group A 158 ± 18; Group B 110 ± 19; and Group C 152 ± 16] were significantly lower when Group B patients were compared with either Group A or Group C. However, no significant differences were found for peak isokinetic quadriceps muscle strength. Therefore, Atenolol, 100mg daily, can significantly lower $VO_{2\ peak}$ and peak heart rate in patients undergoing chronic haemodialysis.

Thus grouping patients with end-stage renal failure who are receiving chronic haemodialysis who receive Atenolol, with patients who do not receive Atenolol, in exercise-related studies may significantly lower $VO_{2\ peak}$ and peak heart rate of the whole group. This lowering may then lead to incorrect conclusions regarding the causes responsible for the low exercise tolerance of patients receiving chronic haemodialysis. Several studies which suggested that low peak heart rate limits exercise tolerance in patients with end-stage renal failure have failed to exclude patients receiving medication from their sample size. Inclusion of these patients may have influenced the results of these studies.

CHAPTER 7

CONCLUSIONS

The exact sequence of events leading to the pathological changes explaining the very low exercise tolerance of patients with end-stage renal failure receiving maintenance haemodialysis has yet to be definitively identified. Opinion is divided between those who believe that the main cause of the poor exercise tolerance in these patients is the reduced oxygen carrying capacity of the blood [Barnea et al., 1980; Kettner, 1982; Zabetakis et al., 1982; Zanconato et al., 1990; Lundin et al., 1991] coupled with low peak exercise heart rates causing impaired oxygen delivery to the active muscles [Moore et al., 1993(a)], whereas others believe that impaired muscle contractile function associated with the renal failure is the primary factor [Kettner-Melsheimer et al., 1987; Robertson et al., 1990]. Recently it has been suggested that in some of these patients, the ability to extract all the physiologically available oxygen from the blood may also limit exercise tolerance in patients with end-stage renal failure undergoing maintenance haemodialysis [Moore et al., 1993(a)].

Hence it is argued that either a central limitation of oxygen delivery to muscle resulting from the anaemia and an associated impairment in oxygen uptake by the active muscles [Moore et al., 1993(a)] or impaired skeletal muscular contractile function, separate from both the anaemia and the postulated impairment in oxygen uptake by the active muscles, explains the poor exercise tolerance of these patients.

On the basis that the mixed venous oxygen content was the same, whereas the arterial-venous oxygen difference ($A\text{-}VO_2$)

and the peak exercise heart rates were less than values measured in other trained and sedentary subjects, Moore et al. (1993(a)) have argued that anaemia together with impaired cardiovascular function and an impaired capacity for skeletal muscle to extract oxygen limit the exercise tolerance of patients with end-stage renal failure undergoing maintenance haemodialysis.

However they argue that the delivery of oxygen to the active muscles is the major factor limiting the exercise tolerance of the majority of these patients.

Furthermore, these authors [Moore et al., 1993(b)] suggest that skeletal muscle weakness cannot be responsible for the low exercise tolerance in these patients because peak heart rates are typically elevated in other groups of patients with skeletal muscle disease and are not reduced as is the case in patients with end-stage renal failure undergoing maintenance haemodialysis.

However there are two findings in this thesis which are not immediately compatible with this finding and hence with the hypothesis of Moore et al. (1993(a)). First if oxygen delivery is the major factor limiting the exercise tolerance of these patients, then the peak blood lactate levels should be elevated. Yet they are low (Tables 3.2, 4.3, and 6.3). Second, there should be a 'plateau' in oxygen consumption during maximal exercise [Noakes, 1988], yet this is also clearly not the case (Chapter 3; Figures 3.1 and 4.1).

Furthermore, Moore et al. (1993(a)) included patients who were on ß-blocker therapy in their sample, and despite acknowledging the effects of this medication on peak heart rates and $VO_{2\ peak}$, they nevertheless concluded that low peak exercise heart rates due to the primary disease process, were responsible for the poor exercise tolerance of these patients.

Moore et al. (1993(a)) also suggest that the exercise tolerance of certain patients with end-stage renal failure undergoing maintenance haemodialysis may be limited by the inability to extract all the physiologically available oxygen during maximal exercise rather than being limited by oxygen delivery.

This theory may help explain our findings that sub-maximal oxygen consumption was identical before and after EPO therapy [Chapter 4]. However, as with the theory that a limited oxygen delivery limits exercise in these patients, this theory can explain neither the low peak blood lactate concentrations nor the absence of a 'plateau' in oxygen consumption during maximal exercise in these patients. Furthermore, it is difficult to conceptualize how patients can switch from one category to the other, that is either oxygen flow limited or oxygen extraction limited, as a result of either EPO therapy or exercise training [Moore et al., 1993(b)].

The critical factor that is ignored by those who argue that oxygen delivery or utilization is impaired, is that oxygen demand is ultimately determined by muscle contractile

activity. If muscle contractile activity is limited by muscle weakness, in particular a specific myopathy of chronic renal failure [Floyd et al., 1974; Chapter 5] or by an impaired central motor capacity to recruit skeletal muscle fibres, then those diseased muscles will be unable to generate sufficient activity to increase oxygen demand sufficiently to tax cardiovascular function. Thus all the evidence provided in support of an oxygen limitation does not disprove the alternate view argued here [Chapter 3] and by other work from this laboratory [Kempeneers et al., 1990], that skeletal muscle function might limit the exercise tolerance of these patients.

Evidence that peripheral skeletal muscle factors could be an important contributor to the poor exercise tolerance of patients with end-stage renal failure receiving maintenance haemodialysis was provided by several important findings from this study.

Firstly, in patients with end-stage renal failure undergoing maintenance haemodialysis, isokinetic muscle strength was a better predictor of exercise tolerance than were variables determining blood oxygen carrying capacity [Chapter 3].

Secondly, the improved exercise tolerance of those patients who receive EPO therapy does not appear to be solely due to reversal of muscle hypoxia which develops during maximal exercise but could result from an effect of EPO therapy on skeletal muscle contractile function [Chapter 4]. The finding

which supports this interpretation is that EPO therapy does not increase oxygen consumption during submaximal exercise even at the maximal workrate achieved before EPO therapy commenced (Figure 4.1).

And thirdly, and perhaps most importantly, we demonstrated morphological changes in the muscles of these patients which may be sufficiently severe to account for the muscle weakness and impaired exercise tolerance found in patients with end-stage renal failure undergoing maintenance haemodialysis [Chapter 5].

However as correctly pointed out by Moore et al. (1993(b)), patients with end-stage renal failure undergoing maintenance haemodialysis terminate exercise with low peak heart rates which is not characteristic of patients with skeletal muscle disease.

At present, therefore, neither theory is able to fully explain the pathological sequence of events leading to the poor exercise tolerance in patients with end-stage renal failure undergoing maintenance haemodialysis. It may, however, be possible that impaired central motor drive or a peripheral neuropathy, or both, may limit skeletal muscle fibre recruitment during prolonged exercise in these patients. Reduced muscle fibre recruitment could explain the low peak heart rates, ventilation and blood lactate concentrations as well the correlation between $VO_{2\ peak}$ and isokinetic muscle strength. Perhaps in this population, isokinetic muscle

testing measures only the ability of the patient to recruit skeletal muscle. Future investigations should, therefore, include EMG activity studies of the lower limb during prolonged exercise in patients with end-stage renal failure undergoing maintenance haemodialysis.

In conclusion, it would appear that the exercise tolerance of patients with end-stage renal failure undergoing maintenance haemodialysis may be limited by central cardiovascular as well as peripheral factors. The most important of these, so far identified, being either a reduced capacity to delivery oxygen to the working muscles, resulting from anaemia and low peak heart rates, or the ability to extract all the available oxygen by the muscles confounded by poor contractile function of the skeletal muscles and decreased muscle fibre recruitment.

Low peak exercising heart rates together with low mixed venous oxygen contents after maximal exercise provide good evidence for a central cardiovascular limitation to exercise in patients with end-stage renal failure undergoing maintenance haemodialysis. But, in addition, profound skeletal muscle weakness which predicts the $VO_{2\ peak}$, low peak blood lactate concentrations, the absence of a 'plateau' in oxygen consumption during maximal exercise, a reduced ability to extract all available oxygen by the muscles toghether with severe morphological abnormalities in skeletal muscle, all indicate a strong peripheral component to the low exercise tolerance of these patients. Thus neither theory appears to

fit all the findings. It is suggested therefore that, in addition to all these abnormalities, skeletal muscle fibre recruitment may be deficient in these patients resulting in low $VO_{2\ peak}$ and peak heart rates, low peak blood lactate concentrations, low peak ventilation volumes, and poor skeletal muscle function.

Invasive techniques to study central cardiovascular function plus other investigations perhaps using electromyography and magnetic resonance spectroscopy to study skeletal metabolism, as well as studies of skeletal muscle contractile function are probably required to identify the predominant causes for the poor exercise tolerance in patients with end-stage renal failure who are undergoing maintenance haemodialysis.

The aim of this thesis will have been served if it helps to direct the attention of renal clinicians and rehabilitation specialists to focus on both the peripheral skeletal muscle factors as well as the central factors, which compromise the exercise tolerance of this particular group of patients.

For the rehabilitation, both physical and medical of these patients, can only be optimised if all the factors limiting their exercise tolerance are addressed.

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